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# PHYSIOLOGICAL REVIEWS

VOL. II

JANUARY, 1922

No. 1

## TRANSMISSION OF PHYSIOLOGICAL INFLUENCE IN PROTOPLASMIC SYSTEMS, ESPECIALLY NERVE

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The general problem of the conditions determining the transmission of physiological influence and control in organisms is one of fundamental interest, since it is evident that the living system can develop, function and react as a whole, *i.e.*, in the unified or integrated manner essential to survival, only in so far as physiological processes in any one region occur in correlation with, or in adjustment to, processes in other regions or in the external environment. Each cell, and in a larger sense each individual organism, constitutes a single self-regulating system, the various separate activities of which mutually influence and control one another; normally the metabolic, formative and functional processes are so coördinated as to secure a definite and constant unity of structure and activity in the whole organism. This unity is apparent at all stages of development, although it exhibits itself in its most striking form in the integration of function and response characteristic of the free-living adult. Obviously any such functional integration implies a ready transmission of influence between the different parts of the living system. In multicellular organisms we now recognize two qualitatively distinct types of such transmission (1). The first depends upon the transport of definite chemical substances from region to region, usually in the circulation; those substances, through their special influence on metabolism or physiological activity, control processes at a distance from their origin (hormone influence; influence of the transportative type (2)). But in addition to this directly chemical type of distance-effect there is a second, evidently of a more fundamental kind, consisting in the rapid transmission of excitatory and inhibitory influence by a process which is independent of direct material transfer; this process is concerned in the majority of responses to stimulation

and attains its highest development in nervous transmission. Motor and sensory reactions, where promptness of response is essential, are especially subject to this type of control. The term "protoplasmic transmission" (3) seems a satisfactory designation, since continuity of living protoplasm—or at least close contact between the protoplasmic structures intervening between the stimulated and the responding areas—seems necessary. Evidently some physiological change in the living protoplasm forms the vehicle of such transmissions.

It is with this second type of transmission that the present article is more immediately concerned. In general, living "irritable" protoplasm is so constituted that the physiological effects produced by local alteration are transmitted more or less rapidly to other regions. All such effects must have a chemical basis. In seeking for inorganic analogies, therefore, a natural comparison is with the "chemical distance-action" (4) well known in polyphasic systems of a certain well-defined type of constitution. Protoplasm represents a reaction-system in which the reacting materials are chiefly in aqueous solution. It is well known that a chemical reaction in one region of a solution may influence another reaction at a distant region of the same solution, without any material transfer between the two regions, provided both reactions occur at the surface of some electrically conducting phase (*e.g.*, metal) which forms with the solution a complete electrical circuit. In any battery the two electrodes together with the connecting wire represent such a conducting phase; in this case the rate of chemical action at the anode is controlled by that at the cathode in the definite manner prescribed by Faraday's law. In general, in any system having the essential constitution of a battery, *viz.*, metal (or other continuous conducting phase) in contact with an electrolyte solution, and with some asymmetry of composition or concentration at two regions of the contact-surface, chemical influence is transmitted instantaneously between the two regions. The essential condition for this transmission is the flow of electricity around the circuit. The electric current can pass between solution and electrode only in association with chemical reactions, which are dependent on the transfer of electrons between the two phases. Since the direction of this electron transfer, relatively to the solution, differs at the two electrode areas, the reactions there occurring exhibit, in addition to the interdependence just defined, a *reciprocity* in their general chemical character; *i.e.*, oxidation at the anode is associated with reduction at the cathode. These considerations also explain why the reactions are confined to the layer of molecules

adjoining the interface, and do not occur in the body of the solution or of the metal.

The protoplasmic transmissions are examples of a process which may be called "physiological distance-action," and in fact various significant analogies with chemical distance-action are apparent, *e.g.*, rapidity of transmission, frequent reciprocity of effect in the two regions influencing each other, susceptibility to electrical influence. Such resemblances suggest that the essential determining conditions in the chemical and in the physiological forms of distance-action are identical and depend on certain fundamental similarities of physico-chemical constitution in the two types of system under comparison.

What we observe in living organisms is that variations of physiological activity in certain regions—implying variations in the rate or character of the underlying chemical or metabolic processes—influence other physiological processes occurring at a distance from the active region. In some cases this influence is of an exciting or initiatory kind (stimulation), in others repressive or inhibitory (inhibition). It may have reference to the most fundamental vital processes, such as growth, or to special activities of the most varied nature. Illustrations of these different effects of transmitted influence are found in all higher animals and plants, and detailed enumeration is unnecessary.

A fundamental feature of this transmissive influence is the reciprocity of action which it often shows in the two regions mutually affected. Acceleration of a physiological process in one region is frequently associated with its retardation or prevention in regions adjoining. This effect is seen with especial clearness in growth-processes. Rapidly growing organs or parts of animals and plants often exert an inhibitory influence on the growth of neighboring parts. A well known instance is the influence of the apical buds of seedlings or of growing branches in repressing the growth of the cotyledonary or axillary buds in adjoining regions. If the apical buds are removed, or if their growth is repressed by cold or anesthetization, the previously inhibited lateral buds sprout out (5). The growth-inhibiting influence thus rendered evident cannot be due to the transport of inhibitory substances from the actively growing zone, as once supposed, since it may be prevented from acting by conditions (cold (6), anesthetization (5), heat-injury (7)) which do not interfere with the flow of materials along the stem. In animals a similar repressive influence is exerted by actively growing or developing or functioning regions; this is best illustrated by the phenomena of regeneration, in which the removal of a part initiates growth and differ-

entiation in regions which formerly were quiescent. Such regions thus show themselves capable of independent growth and differentiation; but apparently in the intact organism these processes are held in check by the influence of the metabolically more active or "dominant" regions adjoining; when removed from this influence—or "physiologically isolated," to use Child's term (8)—they proceed to grow and develop on their own account. In Child's recent books (2), (8) these phenomena of inhibition and dominance in the growth of animals are discussed in detail. Further illustrations of these effects as seen in the growth of vertebrate embryos are to be found in the recent experimental studies of Bellamy (9) and Stockard (10). In the *Fundulus* egg the formation of one actively growing embryonic axis in the blastodisc normally prevents the development of another, so that only a single embryo is formed from each egg; but under certain abnormal conditions (*e.g.*, cold) two such embryonic axes may begin growth; if these are equal in vigor, twinning may result; but if one happens to exceed the other in this respect, it exerts upon the latter an inhibitory influence, with failure of development or regression as a result (10).

According to Stockard (10), the normal sequence of growth and development in the different parts of the embryo is determined largely by influences of this kind. Active proliferation in one embryonic area prevents this process in other areas; when the period of most active growth in one region is passed, another region is released from this restraint and passes through its own cycle of rapid growth, and so on in turn. In Loeb's extensive studies of the growth and regeneration of the plant *Bryophyllum* from detached leaves and stems (11) he describes how the growth of buds on a piece of stem inhibits the growth of roots from a leaf attached to the same stem; he cites various instances of reciprocal influence between the growth of different organs and in general concludes: "if an organ *a* inhibits the regeneration or growth of an organ *b*, the organ *b* often accelerates and favors the regeneration in *a*" (11; *cf.* 1915, p. 276). Such reciprocity is seen not only in the phenomena of growth, but is especially evident in the normal functioning of the central nervous system in higher animals, where the activity of any motor group of neurons represses that of the antagonist group (1). This normal or physiological type of reciprocal influence has an interesting correspondence with the reciprocity exhibited in the effects produced at anode and cathode when a constant current is passed through an irritable living tissue or organism (polar stimulation and inhibition, *electrotonus*, *galvanotropism*), and in all probability has the same essential basis.

Examples of the transmission of inhibitory or excitatory influence to a distance without evidence of reciprocal effects are also frequent. In many animals, both vertebrate and invertebrate, impulses entering the heart through certain nerves (cardio-inhibitory) check or arrest the action of this organ. The propagation of excitatory influence is a more frequently observed phenomenon. In plants Darwin first showed that stimulation of the root-tip, by light or otherwise, modified growth higher up the stem, and many similar instances are now known; and in all animals from protozoa to vertebrata certain forms of local stimulation are observed to initiate, accelerate or modify physiological processes (motor, secretory, etc.) at a distance from the region directly stimulated. In such cases the functional or responding (effector) region is connected with the stimulated (or receptor) region by protoplasmic tracts, differentiated as nerves in higher animals; these conduct or transmit the excitatory influence in the form of waves of chemical and physical alteration travelling at moderate velocities. These velocities are highly variable in different organisms and tissues, ranging from a few centimeters per second or less to maxima of about 100 meters per second in the motor nerves of warm-blooded vertebrates.<sup>1</sup>

Transmissions of this type form the chief subject of consideration in the present article. The essential problem has reference to the general physico-chemical nature of such transmissions, as they occur in all irritable and conductive forms of protoplasm, rather than to the special conditions prevailing in single tissues like nerve. The irritable living substance, wherever found, must have such a constitution that transmission of chemical or metabolic influence to a distance is a constant feature of its activity. Hence the problem of the essential nature of protoplasmic structure or organization is intimately related to the problem of protoplasmic transmission. What kind of structure must any reaction-system have in order to be able to transmit chemical effects to a distance in the above manner? This question leads to a consideration of inorganic systems which exhibit similar powers of transmission, and to a comparison of such systems with living systems, with a view to ascertaining what fundamental features of structure or composition the two types have in common.

<sup>1</sup> In the motor nerves of different animals there is a direct correlation between the velocity of transmission in a nerve and the rate of contraction of the muscle which it innervates (12). Data on velocities are given in many textbooks. Cf. Piper (13) for the special conditions in mammalian nerve, Keith Lucas (14) for frog's nerve.

A simple example of physico-chemical distance-action which bears many suggestive resemblances to the reciprocally acting type of physiological distance-action is seen in the formation of precipitation-structures from metals immersed in solutions of salts the anions of which form insoluble salts with the metal (ferro- and ferrieyanides, carbonates, hydrates, etc.) (15). These effects are well shown in a combination of the two metals, iron and zinc, in an appropriate solution of potassium ferrieyanide. When a strip of zinc foil and a small length of iron wire are placed separately in a watch glass containing this solution (4 per cent  $\text{K}_3\text{FeCy}_6$  plus 0.5 per cent  $\text{NaCl}$ , dissolved in dilute egg-white or gelatine to furnish a protective colloid), each metal reacts characteristically. A precipitate of ferrous ferrieyanide is rapidly formed from the surface of the iron; this precipitate is deposited chiefly in the form of slender blue-green filaments and tubules which elongate rapidly and soon assume a highly characteristic hypha-like appearance; within a few minutes the wire is covered with a dense growth of these precipitation-filaments, some of which may grow later to a length of several centimeters. The zinc reacts with the solution more slowly, and even after some hours usually shows only a few scattered vesicles or filaments of zinc ferrieyanide. If, however, the metals are allowed to react while in contact with each other, *e.g.*, with a strip of zinc an inch long attached at one end to an iron wire of similar length, the reaction of both metals is altered in a striking manner. The formation of filaments from the iron is completely prevented, while their formation from the zinc is markedly accelerated; within an hour the zinc is covered with a characteristic growth of vesicular and tubular precipitation-structures, while the iron remains bright and bare as at first. If now the iron wire is severed with a pair of scissors, the detached piece instantly begins to form filaments, while the still attached part remains unchanged. From this simple experiment it appears that the reaction of the iron is prevented only while it is in metallic connection with the zinc; one might express the facts—using physiological language—by saying that the zinc “inhibits” the growth of filaments from the iron, while the iron “promotes” growth from the zinc. Each metal thus exerts through its contact an influence which modifies the chemical processes at the surface of the other metal, and in a characteristically reciprocal manner. This influence is well marked for a distance of several centimeters from the metallic junction; its intensity decreases progressively with increase in the distance from the contact, and beyond a certain distance the effect becomes inappreciable. Such a gradient of chemical influence

is comparable with the gradient of physiological influence or growth-inhibiting "dominance" seen in developing or regenerating planarians and other animals (2), (6), (16).

The explanation of these chemical transmissions or distance-effects in combinations of metals is simple. Zinc and iron, in contact with each other and with the electrolyte solution, form an electrical couple in which, because of its higher electrolytic solution-tension, the zinc is anode, while the iron is cathode. The direction of flow of the electric current (positive stream) is thus from zinc to solution and from solution to iron; hence the passage of the zinc ions into solution is promoted and that of the iron ions is hindered; this is especially the case where the current intensity is greatest, near the junction of the two metals. Any metal with a lower solution-tension than zinc (Cu, Ag, Pt, Pb, Hg, etc.) resembles iron in its power of promoting precipitate-formation from zinc; conversely the contact of metals of higher solution-tension than iron (Zn, Mn, Mg, Cd, etc.) inhibits the formation of filaments from the latter metal. The precipitate is simply an indicator of the passage of the metallic ions into solution; incidentally it is built up to form a definite type of filamentous or vesicular structure characteristic for each metal. The rate of the reaction and the distance through which the chemical influence is perceptible depend essentially on two factors, (a) the potential-difference between the two metals, and (b) the electrical resistance of the solution. Obviously the intensity of the current passing between metal and solution at any point on the surface of either metal must fall off progressively with increase in the distance from the contact of the two metals, since the electrical resistance of the circuit which includes the point considered depends chiefly on the length of the column of solution between that point and the metallic junction. Hence near the junction the mutual influence of the two metals is greatest, and beyond a certain distance it ceases to be perceptible. Both the transmission of the chemical influence to a distance and the existence of a gradient of such influence are thus directly dependent on the electrical nature of the chief factors.

It appears probable that certain forms of physiological distance-action, especially the growth-inhibiting and other reciprocally acting influences cited above, are to be referred to general conditions of the kind just described. Electrical potential-differences have long been known to exist between actively growing regions and the less active regions (17); hence currents must flow in definite directions through the growing organism (18); and it is also known from the facts of galvano-

tropism that electric currents influence growth. According to the present hypothesis, those cases where there exists a well-defined gradient of growth-determining influence fall in the general category of effects illustrating the influence of the electric current on growth. Already there is much evidence indicating that in general actively growing or regenerating regions of an organism are negative (like physiologically active regions in other tissues) relatively to quiescent regions (19), (20), (21); *i.e.*, the direction of the bioelectric "growth currents" (positive stream) is from medium to living protoplasm in the growing regions and from protoplasm to medium in the adjoining quiescent regions. In these two regions the currents have opposite directions relatively to the cell-surfaces; and to this difference of direction must correspond a difference in the influence on growth, since the constant current always exhibits polarity in its physiological action. This is presumably the basis of the reciprocity described above. Whether the conditions of local current-intensity, resistance, and susceptibility to weak currents correspond to the requirements of the above theoretical conceptions in all details can be determined only by future experiment. At the present time further and more exact work on the effects of the electric current on growth is much to be desired.<sup>1a</sup>

Transmissions by means of the direct influence of bioelectric currents, exerted under conditions similar to those of the above metallic models, *i.e.*, where the intensity of the transmitted effect necessarily decreases with distance from the physiologically active or dominant region, cannot, however, form the basis of the rapid forms of excitation and transmission exhibited in nervous and muscular action. In nerve especially the transmitted influence shows no decrease in intensity as the distance from its origin increases; this is shown clearly by the case of "trapped waves" in rings of medusa tissue (22). A self-propagating wave of excitation, associated with a local bioelectric circuit, passes along the conducting tissue and arouses physiological activity at all points along its path and in the terminal organ (phenomena of conduction and innervation). Yet here again simple types of inorganic

<sup>1a</sup> While the present review is in proof, an experimental study by Lund (99) on the electrical control of regeneration in the cut stems of the hydroid *Obelia* is announced as about to appear in the *Journal of Experimental Zoölogy*. The formation of new hydranths is promoted where the positive stream of a weak current enters the cut end of the stem, and inhibited where it leaves. Sven Ingvar (100) has recently obtained polar directive effects of a similar kind in studying the influence of weak constant currents on the outgrowth of the processes in embryonic nerve-cells.

process are known which simulate this second form of protoplasmic transmission in many of its most characteristic details.

Nervous transmission is the physiological type-phenomenon of this class. In all such phenomena the essential effect is the spreading of a certain active state (23); this state is initiated by some local change caused usually by some external agent or "stimulus". We may thus distinguish between (a) the local effect produced by the stimulating agent at its point of application and (b) the physical or chemical disturbance there set up which is propagated to a distance (conducted effect) (24). This "propagated disturbance"<sup>2</sup> (activation-wave, nerve-impulse, etc.) has the property of altering physiological activity wherever it extends, *e.g.*, of stimulating or inhibiting activity in the terminal organ. The essential problems therefore are: why it should be propagated, or spread from region to region along the conducting tissue, and why it should initiate or alter physiological activity in the same tissue or in another tissue (*e.g.*, muscle) to which it is transmitted.

There are also forms of transmission—apparently intermediate between the two types above distinguished—in which a conducted influence is transmitted through an often considerable distance, but with a progressive decrease in "intensity" or physiological effectiveness as it travels. This is the case of "conduction with decrement" (25). Certain normal types of physiological gradient seem to depend on transmission of this kind, as indicated by the fact that the influence may be intercepted by cold or anesthetization (5), (6). In the central nervous system it appears to play an important part (14); thus the penetration of the impulses determining sensory impressions, association processes, or reflexes in the brain or spinal cord is limited and varies with the physiological state (sleep, fatigue, etc.) This decrement type of conduction can be artificially induced in nerve or muscle by cold, fatigue or anesthesia. For example, an impulse initiated in a normal region of a nerve penetrates at a lessened velocity for a certain distance along an anesthetized stretch, but is eventually extinguished if the latter is of sufficient length; if, however, the impulse succeeds in traversing this "region of decrement" and emerges into the normal region beyond, it there regains its original velocity, amplitude and other properties (26) (see below, p. 30). If "intensity" is measured by the ability to penetrate a region of known decrement, as Lucas has proposed (14), it is

<sup>2</sup> Keith Lucas' term; for the experimental basis of the distinction between local and propagated effects *cf.* Lucas and Adrian (24a).

evident that this property progressively decreases as the impulse travels along such a region.

In any transmission without decrement, such as we find in a normal motor nerve, the process occurring at any stimulated region of the transmitting element initiates in adjoining regions a process which is similar qualitatively and quantitatively to that at the original region; and each region thus secondarily activated influences the region beyond in the same manner. In a uniformly constituted elongated element of this type propagation for an indefinite distance at a uniform speed is to be expected; this corresponds to what we observe in nerve. Conditions of an analogous kind are seen in the transmission of a wave of combustion along a fuse; at any instant there exists for a certain distance in advance of the burning region a certain gradient of temperature; within a portion of this gradient the temperature is above the ignition-temperature; and the speed of travel depends on this critical distance and on the rate at which the heat-generating reaction proceeds (*i.e.*, on the local reaction-velocity). In the case of the conducting protoplasmic element the transmission from the active to the adjoining resting region is apparently a result not of the establishment of a difference of *temperature*, but of a difference of *electrical potential*. In other respects, however, the analogy is an instructive one. According to the present conception, up to a certain distance in advance of the active region ( $R_4$ , fig. 1) the current of the local active-resting circuit is sufficiently intense to cause secondary electrical excitation; the length,  $s$ , of this critical distance, and the rate,  $v$ , at which the variation of potential rises to its maximum in the active region (a measure of the local reaction-velocity) determine the speed of propagation,  $P$ . Expressed algebraically,  $P = Ksv$ , or  $P = Ks t$ ,  $K$  being a proportionality-factor and  $t$  the time occupied by the variation of potential (27). There exists in fact a close proportionality between the rate at which the bio-electric variation rises to its maximum in different conducting tissues and the rate of transmission of the excitation-wave (28).

Inorganic systems exhibiting this form of transmission, *i.e.*, transmission by means of the electrochemical effects produced near the boundary between altered and unaltered regions, are well known, and usually consist of oxidizable metals immersed in electrolyte solutions containing an oxidizing agent which acts energetically enough to form a continuous film of oxidation-product over the whole surface of the metal. Mercury in hydrogen peroxide (29) and passive metals, especially iron, in strongly oxidizing solutions like nitric acid are the

best known examples (30). The spreading action depends upon the formation of local circuits between the film-covered and the free metallic surfaces; through the electrolytic action of these circuits the film is locally removed; and this effect is automatically self-propagating, since wherever film-covered and free metallic surfaces adjoin each other a similar circuit is formed and the effect is repeated. That phenomena of this type exhibit in many cases remarkably close analogies to physiological processes (in rhythm, automaticity, conduction, etc.) has been recognized by a number of investigators. Bredig and his students have investigated the conditions in the  $\text{Hg-H}_2\text{O}_2$  system (31), although without special reference to the problem of protoplasmic transmission; and the transmission of the activation-wave over the surface of passive iron in nitric acid—a phenomenon with especially striking physiological analogies—has been compared to nervous transmission by Ostwald (32), Heathcote (32) and others (33).

Recently I have studied in some detail (3), (33) the analogies of this phenomenon to protoplasmic transmission of the nervous type, and have endeavored to account for the above similarities on the basis of the fundamental features of structure and activity possessed in common by all polyphasic systems having thin chemically alterable interfacial films (34). The presence of films deposited at the interphase surfaces determines the properties of many such systems, *e.g.*, the stability and physical consistency of emulsions and certain types of gel.<sup>3</sup> Local variations in the chemical composition, permeability or other properties of these films should theoretically give rise to potential differences between adjacent areas; and under certain conditions it is conceivable that local electric currents may thus arise which secondarily may influence chemical processes in the system. Since living protoplasm is in fact a film-partitioned system, and since many irritable cells are known to be bounded from their media by semi-permeable membranes whose composition and properties vary with the physiological state of the cell, it is perhaps not surprising that protoplasmic systems should exhibit transmission-phenomena of a type closely similar to those found in film-covered metallic systems such as passive iron in nitric acid.

The phenomena of passivity in metals are well known (30), and only a brief account of the more relevant facts need be given here. An iron wire dipped in strong nitric acid or other suitable oxidising agent and then transferred to weaker acid (sp. gr. 1.20, in which the normal or

<sup>3</sup> For the structure and properties of emulsions and related systems *cf.* the very full review of W. D. Bancroft, *The Theory of Emulsification* (35).

"active" metal dissolves rapidly) shows no reaction; the metal is in the so called "passive" state, but it may be made to react (or "activated") by touching with ordinary active iron or other base metal, or by mechanical means (bending, scraping with glass, etc.). The reaction, which is accompanied by effervescence and the production of dark-colored lower oxide, then sweeps over the whole surface of the metal, at a rate which varies with the conditions but may attain some hundred centimeters per second, a velocity of the same order as rapid protoplasmic transmissions. Of especial interest from the physiological standpoint is the fact that in  $\text{HNO}_3$  of more than a certain concentration (sp. gr. ca. 1.24) the reaction is temporary, the metal reverting spontaneously to the passive state; after an interval—more or less prolonged according to the concentration of acid—during which reactivation is difficult or partial (because of imperfect transmission) the metal can be reactivated as before (37).

It is now generally agreed that passivity is due to the presence of a thin, coherent and impermeable surface-layer of oxidation-product (probably higher oxide), and that during activation this layer is removed by electrochemical reduction near the boundary between the active and the passive regions. Any region of a passive wire may be made locally active by removing or interrupting the passivating film, mechanically, chemically or otherwise. Such an active region represents the anode of the local couple thus formed; at the adjoining still passive or cathodal area the surface-film is reduced and disintegrated; this region then becomes active in its turn, *i.e.*, anodal, and the same sequence of effects is repeated at the regions beyond. Hence a wave-like spread of activity over the whole surface follows any sufficient local alteration. Wherever the surface-film is removed, interrupted or rendered permeable, the metal at once reacts with the acid. In repassivation the film is restored and the reaction ceases. The reason why this repassivating reaction occurs automatically in strong  $\text{HNO}_3$  is that each region as it becomes active becomes also anodal, and is hence subjected to the anodal oxidizing influence, in addition to that exercised by the  $\text{HNO}_3$  itself. In general, the variations in the reaction of the metal with the acid thus depend on variations in the film-structure of the system (33). Under certain conditions these variations are rhythmical, and the film undergoes regular and automatic alternate formation and dissolution, giving rise to a rhythm of reaction resembling in its time-relations and other features physiological rhythms like that of the heart-beat. A similar rhythm is shown by mercury in  $\text{H}_2\text{O}_2$  (31).

The essential features exhibited in common by transmission in passive iron and in nerve (or similar protoplasmic systems) may be summarized as follows. 1, A local or initiatory process may be distinguished from a propagated disturbance or wave of reaction. Local alterations of various kinds, mechanical, chemical or electrical, may initiate an activation-wave in either system; once initiated its propagation is automatic and determined by the state of the transmitting wire or filament and of the adjoining solution. The "all or none" character of the reaction in readily transmitting metallic or protoplasmic systems is thus explained. 2, The local reaction accompanying the passage of the reaction-wave is automatically reversible, but there is always a certain delay (called "refractory period" in the living system) before the system returns to its original irritable and conductive state. (In the passive metal this interval is that required for the formation of a surface-film with the original properties (33).) 3, In a uniform nerve fiber or passive iron wire the activation-wave shows the same characters at all points along its path. It is associated with a variation of electrical potential, and with a temporary loss of continuity or breakdown (increase of permeability) in a surface-film of chemically alterable material. This latter change is demonstrable in passive iron and in certain slowly conducting protoplasmic systems (*e.g.*, echinoderm egg-cells during the fertilization-reaction (36)), but in nerve the evidence for its existence is indirect. 4, The propagation-velocities in both systems are of the same order (ranging from a few centimeters to some meters per second), and exhibit a high temperature coefficient (of the order of  $Q_{10} = 2$ ). 5, In both systems the activation-wave may be retarded or blocked by certain forms of chemical or electrical influence, *e.g.*, electrical polarization (anelectrotonus in the living system, anodal polarization in the passive iron system (37, p. 136)) or chemical alteration (*e.g.*, narcosis in the living system). 6, Interruption of metallic or protoplasmic continuity prevents the passage of the wave, but activation may be transmitted by contact. This is shown in the living system by certain structural features in the arrangements of neurones in the central nervous system (synapses, end-feet), by the characters of the motor end-plates and other nerve endings, and by the facts of secondary stimulation by bioelectric currents (rheoscopic nerve-muscle preparation, etc.).

The conditions under which a local alteration ("stimulus" in the living system) initiates the activation-wave also show many close parallels in the two systems.<sup>4</sup> The chief of these are as follows: 1,

<sup>4</sup> For the condition in the passive iron model *cf.* Science, 1918 (33).

A certain intensity or degree of local alteration is required ("threshold"); too slight a mechanical or electrical disturbance will not start a wave of activation, although it may produce a temporary local effect,—shown by temporary change of potential in the wire, or by the fact that in both cases a sufficiently rapid succession of such subminimal effects will activate (37, p. 134). 2, A succession of local mechanical or electrical "stimuli", each of which singly is inadequate, will start an activation-wave if the interval between the stimuli is sufficiently brief (summation effect).<sup>5</sup> 3, Activation by the electric current is a characteristically "polar" effect; the passive wire is activated when it is made cathode in a circuit, but not when it is made anode; in the latter case activation by another agent (*e.g.*, mechanical) is rendered more difficult during the flow of the current (37, p. 136). In the living system the parallels are polar stimulation and polar inhibition (or anelectrotonus). 4, Too weak an electric current will not activate, no matter how long it is continued (critical or threshold intensity), and a current of sufficient intensity for activation must flow for more than a certain time in order to produce its effect (39). 5, A current rising too gradually from subminimal to a sufficient intensity will not cause activation; *i.e.*, more than a certain critical rate of change is required in the activating current (40).

Conditions 2, 4 and 5 show that both types of system have an automatic tendency to preserve a certain state—corresponding to the passive or resting state—and to resist displacement from this equilibrium; the disturbing or activating condition must therefore act for a sufficient time and more rapidly than the automatic passivating counter-changes in order to bring the local alteration to the level required to initiate an activation-wave.<sup>6</sup>

Evidently the local alteration or stimulus originates a condition or process which is automatically self-propagating. The originating external agency, *e.g.*, a mechanical impact, produces at its point of application some surface-alteration which is (or may be) quite different from the propagated reaction which follows; its immediate effect, however, appears in all cases to be the establishment of a certain critical

<sup>5</sup> A brief qualitative account of mechanical summation in the passive iron model is given in my recent paper (37, p. 134). Summation can also easily be shown for electrical activation, using, *e.g.*, the contact of a copper wire to produce a brief local current. For an exact study of the summation-interval in living tissues *cf.* Keith Lucas (38).

<sup>6</sup> *Cf.* Hill (43) for a discussion of the possible conditions in the living tissue.

potential-difference between the region thus altered and the unaltered region adjoining. In the metallic model the existence of this effect may be shown experimentally by activating mechanically a passive iron wire which is immersed in  $\text{HNO}_3$  and connected through a galvanometer with an indifferent electrode (*e.g.*, platinum wire) immersed in the same solution. An ineffective scrape with a glass slide causes a slight temporary excursion of the instrument, indicating a quickly reversed change of potential, while an effective scrape or succession of scrapes causes a larger excursion which is at once followed by that accompanying the full propagated reaction (37, p. 134). In the living system something analogous seems to occur; apparently with the rapid establishment of the necessary P. D. between stimulated and non-stimulated areas, and hence of a local bioelectric current, the condition for-propagation arises.

It is a familiar fact that the same irritable tissue (*e.g.*, nerve) may be stimulated by many different agencies, mechanical, electrical, thermal, chemical, osmotic; *i.e.*, all originate the same self-propagating state of excitation. Why this should be the case becomes clear on the theory that in all forms of stimulation the electrical factor is the essential. Any sufficient mechanical, chemical or other alteration of the cell-surface is known to produce a local electrical negativity, as may readily be shown in muscle and nerve; a local bio-electric current (injury current, alteration current) arises between the altered region and the unaltered regions beyond. If we assume that the spread of the excitation-state, and with it the stimulation of the irritable element as a whole, depends primarily on the effect produced by the local electric current formed at the original area of stimulation, the whole problem at once assumes a more definite as well as simpler aspect. It resolves itself into the problem of the conditions of electrical stimulation in general. The current passing between the locally altered area and the unaltered area adjoining initiates "secondarily" electrical excitation in the latter region; and since any excited region becomes also locally negative, a similar current at once arises between this region and the one immediately beyond, which is then similarly excited. By a repetition of this effect at each newly formed active-resting boundary the excitation-wave travels over the entire irritable element, and the whole system is activated.

The problem of electrical stimulation can be considered only briefly in this article, which is concerned with the general conditions of transmission rather than with the special nature of the local effect produced

by the current. It is now agreed that a change in the electrical polarization of the limiting semi-permeable boundary-layer of the irritable cell or element (protoplasmic surface-film or plasma-membrane) constitutes the primary change in electrical stimulation. This theory has been established on a firm foundation by the work of Nernst (41) and his successors, especially Lapicque (42), Lucas (39) and Hill (43); and the quantitative conditions of electrical excitation are considered in detail in the papers of these authors. The results of this and related work have shown that the current effects local stimulation 1, when it has a definite *direction* relatively to the cell-surface, such as to effect a diminution of the preëxisting or physiological polarization;<sup>7</sup> 2, when it has more than a certain minimal (threshold) *intensity*; 3, when it flows for more than a certain minimal *time*; and 4, rises to its full intensity at more than a certain *rate*. The essential condition for electrical stimulation is thus a critical change of polarization (a depolarization (44)), to produce which requires a certain minimal intensity, duration and rate of change in the stimulating current.

These latter conditions differ in a significant manner from those required to produce a given change of polarization (*i.e.*, to establish a given P.D.) across a non-living partition set in the path of a current, the case considered by Nernst (41). In this case a weak or slowly increasing current, if it lasts long enough, may produce the same change of polarization as a stronger current lasting for a brief time, a condition formulated in Nernst's "square root law" ( $P = Ki\sqrt{t}$ ).<sup>8</sup> The difference between the dead and the living system indicates that in the membranes of the irritable tissue processes are acting whose general direction or effect is such as to oppose or compensate the processes set in action by the stimulating current; normally the effect of these compensatory processes (presumably processes of metabolic construction, at least in part) is to bring the tissue back to the resting state after stimulation and to keep it in the normal irritable state in the intervals between stimulation. The stimulating agency must act at a greater rate than these compensatory processes, and last long enough to offset their effect, or no stimulation results.<sup>9</sup> A condition

<sup>7</sup> This is the necessary inference from the law of polar stimulation (excitation at cathode, inhibition at anode on make of constant current, and *vice versa*), as pointed out by Brunings (44).

<sup>8</sup>  $P$  represents polarization produced,  $i$  the intensity and  $t$  the duration of the polarizing current.

<sup>9</sup> Compare the discussions of Lapicque (42) and Hill (43) on the nature of the conditions necessitating a certain minimal rate of change in the stimulating current.

of this general kind is found also in the passive iron wire in strong nitric acid, where the oxidizing action of the acid keeps the passivating surface-film continuous and automatically repairs slight interruptions (*i.e.*, those insufficient to start activation-waves); the same rapidly acting oxidative process also reforms the film after the passage of an activation-wave. In this system also a rapidly increasing current of more than a certain duration is required for activation; in fact the above four rules of electrical stimulation apply also to the case of electrical activation of passive metals (33).

Apparently any rapid local decrease of surface-polarization (sufficient in range) causes stimulation in the typical irritable system such as muscle or nerve. This purely physical change, however, is merely the precursor or determinant of the local stimulation-reaction; it is not the reaction itself. The latter is a physiological process dependent, like all such processes, on chemical reactions, and having its own special peculiarities (rate, duration, chronaxie, specific features) in each irritable tissue (39), (42). The general problem is why this process should be initiated by a change of electrical polarization at the cell-surface. The only general inorganic analogy is that of electrolysis. In this case the establishment of a sufficient uncompensated potential-difference between electrode and solution forms the condition of the chemical change. When the electric current is free to pass (*i.e.*, with closed circuit) there is transfer of electricity (electrons) between electrode and solution; at the interface electrons are added to or abstracted from the molecules or ions there present, with chemical combination or decomposition as a result. We have seen that in the metal-electrolyte combination regarded above as affording a generalized model of protoplasmic transmission—passive iron in nitric acid—a local electrolysis, resulting, *e.g.*, from the contact of zinc or from a mechanical interruption of the oxide coating, forms the primary condition of a rapidly propagated wave of chemical and electromotor change also depending on electrolysis; and attention has been called to the many significant resemblances between this process and protoplasmic transmission. The implication is that in the transmitting protoplasmic system also the local bioelectric current produces, by a process essentially identical with electrolysis, chemical alterations in the surface-film, and that the general tendency of excitation-processes to spread in living protoplasm is a result of this condition, which is essentially similar to that existing in the inorganic model. At present the special chemical conditions at the protoplasmic interfaces are unknown, but in their general features the

two processes give unmistakable evidence of belonging to the same physico-chemical class.

According to this conception, protoplasmic transmission is an electro-chemical effect, depending on the formation of a circuit between the altered area and the adjoining unaltered area. For a certain distance (e.g.,  $R_3 R_4$ , fig. 1) beyond the boundary between these areas the current of the local circuit is sufficiently intense to effect the required electrolytic alteration of the surface layer; in the case of the passive wire this alteration consists in the reduction of the passivating oxide film to a lower state of oxidation; in the case of the protoplasmic system its precise nature is unknown. Any such effect is self-propagating because between the newly altered area and the area beyond a similar circuit is formed, and the same effect is repeated at each new boundary. Since the essential condition for propagation is alteration of the interfacial

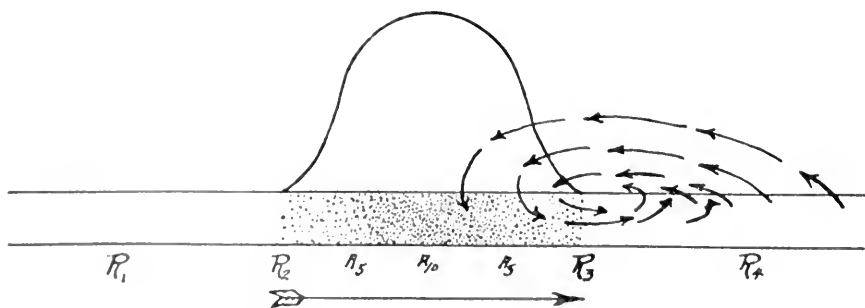


Fig. 1. Diagram of the momentary conditions in a frog's motor nerve at 20°. The shaded region marked  $A$ , between  $R_2$  and  $R_3$ , is occupied at the instant under consideration by the excitation-wave, which is regarded as advancing in the direction of the large arrow at the rate of 30 m. per second. Its length is 6 cm., assuming the total duration of the local process (as indicated by the duration of the local bio-electric variation) to be 0.002 second. The excitation-process is just beginning at  $R_2$ , has reached its maximum at  $A_{10}$ , and has just subsided at  $R_3$ . The curve indicates the variation from the resting potential at different points in the active region; the maximum P. D., at  $A_{10}$ , is 30 to 40 millivolts. The unshaded regions marked  $R$  are in the resting state. The small arrows indicate the general direction of the bio-electric current (positive stream), in the external medium and in the protoplasm, in a portion of the circuit at the active-resting boundary. For a certain distance beyond the boundary ( $R_3 R_4$ , probably about 3 cm.) the intensity of this current is sufficient to excite the nerve; excitation is thus in process of initiation in the still resting region of the nerve for this distance in advance of the wave-front. For a somewhat similar distance  $R_1 R_2$  in the wake of the excitation-wave the nerve is refractory to stimulation.

film at some distance from the already altered area, by means of the electrochemical action of the local current, it is evident that the possibility of transmission depends in the first instance upon the "chemical distance-action" effect (15, p. 165). As each new active-inactive boundary is formed the same situation arises; there is always, therefore, extending for a certain distance in advance of the already altered region a region within which the electrochemical reduction is in progress. The length of this interval, and the rate at which the film is electrochemically removed or altered, are the two chief factors determining the velocity at which the reaction is transmitted along the surface (27), (28).

This general description is based more particularly upon the conditions in the passive iron system, where the chemical conditions of transmission are comparatively simple and readily understood. In the case of a protoplasmic system like nerve, the precise nature of the chemical reactions determining transmission is unknown. There is little or no change of temperature accompanying the passage of a nerve impulse (45), but the significance of this fact is obscure;<sup>10</sup> possibly heat-production and heat-absorption balance each other in the local reaction. It seems probable that oxidation-reduction processes are concerned in many transmissions, and in this case the local bio-electric circuit may represent essentially an oxidation-reduction circuit.<sup>11</sup> The majority of stimulation and transmission processes are directly or indirectly dependent on oxygen-supply; but whether molecular oxygen enters into the local reaction in its initial destructive phase, or in its later reconstructive or synthetic phase, is uncertain. More probably oxygen is especially required in the construction phase, during which the altered surface-film is renewed (37). Nerve is not exhausted by stimulation except in the absence of oxygen (46a), (47); this condition is also known

<sup>10</sup> It cannot be explained until more is known of the nature of the local current-producing reaction. It is well known that certain electrochemical combinations abstract energy from the surroundings; according to Bernstein and Tschermak this is also true of the electric organ of the torpedo (*cf.* Bernstein, 86, chapter 6). The energy of the local bio-electric current in a nerve is insufficient to produce an appreciable change of temperature (*cf.* Hill, 48).

<sup>11</sup> There is some evidence (regarded as inconclusive by many physiologists) of an increased production of  $\text{CO}_2$  by nerve during activity (*cf.* Waller 45a, Tashiro 45b). The absence of any increased rate of change in the pH of the medium bathing the nerve during activity, as reported by Moore (46) is not necessarily inconsistent with this result, since there is the possibility that basic compounds (*e.g.*  $\text{NH}_3$ ) may be freed from the nerve at the same time.

to prolong the refractory period (47), which apparently represents the period of reconstruction of the surface-film (37). In general, the above analogies with the metallic system point to the conclusion that the essential process in the local excitation-effect consists in a breakdown of the protoplasmic surface-film, under the electrochemical influence of the local bio-electric current, followed by its reconstruction under similar influence,<sup>12</sup>—which becomes oppositely directed as soon as the direction of the local “diphasic” current changes.<sup>13</sup> The process is

<sup>12</sup> The terms “breakdown” and “reconstruction” may seem to imply a more thorough-going alteration than actually occurs in the surface-film of the irritable element during the passage of the excitation-wave; but in our ignorance of the details of the process they may be regarded simply as indicating the general character of the two phases of the reversible or cyclical process at the excited area. Apparently the breakdown (“catabolic” change) corresponds to the rising phase, the reconstruction (“anabolic” change) to the return phase of the electric variation; on this view the physiological conditions have a general correspondence with those in the metallic model (compare the earlier view of Hering: *Theory of the Functions in Living Matter*, Lotos, ix, Prag, 1888, translated in *Brain*, 1897, xx, 232).

<sup>13</sup> Some light appears to be thrown on this problem by the effects of change of temperature. It is probably significant that the temperature-coefficient of transmission in muscle and nerve is definitely lower than that of the refractory period, which represents the time occupied by the process of restoration immediately following the passage of an excitation-wave. According to the recent results of Adrian (49), the temperature-coefficient of the rising phase of the bioelectric variation also appears to be distinctly less than that of the declining phase. Now the rising phase of the electromotor variation, in both the passive iron model and the living tissue, is determined (on the present evidence) by the breakdown (or increase of permeability) of the surface-film. Transmission is a direct consequence of this effect, as already indicated. The return or declining phase (in which the passive or resting potential returns) represents the period in which the surface-film is reformed. The results of Maxwell (50), Snyder (51), Lucas (52), Woolley (52a) and Harvey (52b) all indicate a  $Q_{10}$  of about 2 for the transmission-rate in muscle and nerve; while for the refractory period the most exact determinations (Bazett (53), Adrian (49), (51)) give values of 3 or more, a coefficient similar to that of growth processes.

On the theory that transmission is dependent on breakdown of the surface-film, and recovery on its reconstruction, it seems possible to explain this difference. Reconstruction is essentially a matter of metabolic synthesis and should have a purely chemical temperature-coefficient; while breakdown is probably mainly the result of physical disintegration, following some initial chemical alteration. Let us assume that the total period of breakdown at 20° has a duration of  $3\sigma$ , and that one-third of this period ( $1\sigma$ ) is occupied by the initial chemical decomposition ( $Q_{10} \approx 3$ ) and the remaining two-thirds by a physical disintegration (with  $Q_{10} \approx 1.3$ , the value for diffusion-processes). The duration of the total process at 10° would then be  $1\sigma \times 3 + 2\sigma \times 1.3 = 5.6\sigma$ . The ratio of the

self-propagating because in a film-covered system like protoplasm it cannot occur without the production of potential-differences, and hence of local circuits which produce chemical effects involving similar alterations of film-structure in regions adjoining.

In considering cases where the detailed nature of the chemical processes is obscure, as in nerve, a more generalized conception of the above general type of situation may be desirable. Let the two parallel lines in figure 2 represent the contour of a filament, wire, or other conducting structure, covered with a chemically alterable surface-film of uniform composition and immersed in an electrolyte solution. So long as the system is undisturbed, the conditions at any two points, *e.g.*, *A* and *B*, are chemically and electrically symmetrical; the interfacial P.D. is the same at all portions of the surface, hence no current flows through the circuit if the two points are connected through a galvanometer. But if now one region (*e.g.*, *A*) is altered—chemically, mechanically, thermally, or osmotically (*i.e.*, by a concentration change)—the



Fig. 2

two regions are no longer symmetrical and a current flows; if this current produces through its electrochemical effect an alteration at *B* corresponding to that at *A*, transmission will result.

It is evident that such a generalized schema may apply to systems of the most widely varying chemical composition. The details of the conditions in any system like nerve can be determined only by special

velocities at the two temperatures, 5.6/3, or 1.9, is similar to that observed for transmission velocities in nerve. Thus if transmission is due to a disintegration effect, following a brief initiatory chemical effect, a low temperature-coefficient is to be expected.

A further indication that the two processes are essentially different in character is seen in Lucas' observation (55) that a nerve may exhibit a normal rate of recovery in solutions of alcohol which greatly retard transmission. Transmission and recovery are thus differently affected by both temperature and chemical reagents. It is known that the refractory period may be artificially lengthened by conditions (poisons and lack of oxygen) which have little effect on transmission or on the rising phase of the bio-electric variation (93), (94), (95). In the passive iron model the duration of the recovery phase may be varied within wide limits without affecting the rate of transmission, by simply varying the concentration of the  $\text{HNO}_3$  (37).

investigation; but it is to be remembered that the possibility of transmission of the type under consideration is determined by the *general* features of the system and not by its special peculiarities of composition and structure. The essential condition in all cases is the presence of a thin, chemically alterable surface-film separating two media, both of which conduct electricity, the passage of electricity between the two media being attended with chemical and structural alteration in the film.

There is in fact much evidence that stimulation processes are accompanied by alteration or temporary breakdown (associated with permeability-increase)<sup>14</sup> of protoplasmic film-structure, especially of the limiting surface-films or plasma membranes (56), (57), (27). The fundamental theoretical importance of such evidence will be clear from the foregoing, and a brief review of the chief known facts will indicate how widespread such processes are in living protoplasm. In its elementary physico-chemical constitution protoplasm is most closely related to the emulsions or emulsion-like systems, as Bütschli recognized many years ago (58); and, as in other such systems (35), interfacial films are undoubtedly a chief factor in determining its normal physical properties and especially its structural stability, *i.e.*, in preserving the normal subdivision and distribution of the component phases (59), (60). In many instances, the protoplasmic emulsion is highly unstable. The platelets of mammalia and the "explosive corpuscles" of crustacea (61) are well-known instances; here slight chemical or mechanical changes lead to a rapid and complete disintegration of protoplasmic structure. Many striking instances of this type of effect have been brought to light since the introduction of the methods of micro-dissection. Leucocytes, red blood-corpuscles, germ-cells and other cells not ordinarily classed as "irritable," when cut or punctured locally by a capillary needle, often exhibit a spread of disintegrative alteration which may involve the whole protoplasm (62). The protoplasmic boundary-film loses its normal semi-permeable properties, and this effect spreads; a good illustration is Chambers' observation that a red corpuscle pricked at one point immediately shows loss of hemoglobin over its whole surface (63). Recently many interesting instances of progressive disintegration of protoplasmic structures, including membranes, following local injury have been observed in Protozoa by Taylor (64). In other cases the injury may remain

<sup>14</sup> A breakdown would imply interruption of continuity and hence increase of permeability, of which there is much evidence (56).

localized, its spread being delimited by the formation of a new surface-film; this phenomenon is frequent in echinoderm egg-cells (65). The spread of such disintegrative effects varies according to the conditions, *e.g.*, with the extent of the local injury or the state of the protoplasm. In the isolated nerve-ganglion cells of the lobster Chambers found that more than a certain degree of mechanical injury resulted in a complete and irreversible change in the properties of the protoplasm. "When this limit is passed the viscid plasma sets into a coagulated non-viscous mass which may be broken into non-glutinous pieces" (66). Loss of mechanical coherence, usually associated with coagulative changes in the cell-proteins, is a frequent effect of irreversible protoplasmic alteration of this kind; this is seen, *e.g.*, in the death-rigor of muscles. A similar change occurs as a result of excessive stimulation in certain irritable and contractile forms of protoplasm, *e.g.*, the large compound cilium or swimming plate of ctenophores (67). When these structures are placed in certain solutions (*e.g.*, pure isotonic NaCl) there follows a marked and striking acceleration of contractile or vibratile activity during which the normally clear and translucent protoplasm undergoes a progressive whitening or coagulation; both processes cease within a minute or two, by which time the protoplasm has lost its structural coherence and has become permanently opaque and coagulated. Evidently the physical state (subdivision, etc.) of the structural proteins is irreversibly changed as a result of some process associated with intense stimulation. It is especially noteworthy that during the excessive activity resulting from this treatment the individual fibrils or cilia composing the plate frequently lose coherence and vibrate independently. Apparently the normal interstitial material, corresponding to an interfibrillar film-structure, is irreversibly broken down during the continued excessive activity resulting from the abnormal stimulation. It is to be presumed that during the normal rhythm of contraction this film-structure is alternately broken down and replaced in such a manner that the restoration during each contractile cycle is complete, but that under the above abnormal conditions the process of reconstruction is imperfect and progressive disintegration results.

The ability of cut or injured protoplasmic surfaces to form new film-structure has long been known; this property is highly significant in relation to the power of repair and maintenance possessed by all forms of protoplasm. In the recovery of the irritable cell or element after stimulation this film-forming process is almost certainly concerned, as indicated especially by the phenomena of the refractory period

above referred to; in certain egg-cells (*Echinarachnius*) a process of apparently the same kind can be shown to occur immediately after insemination (36). In those cases where the breakdown of the protoplasmic structure is more rapid than can be compensated by the succeeding reconstruction the effects of stimulation may be irreversible; this appears to be exemplified in the above cited instances of the lobster ganglion-cell and the ctenophore swimming-plate; structural breakdown from exhaustion is in fact well known in higher as well as in lower organisms.<sup>15</sup> A recent review by Seifriz (69) gives many instances of this capacity of living protoplasm to form new films or plasma membranes at cut or altered surfaces.

In any irritable and conducting cell or element altered by normal stimulation, the formation of a new surface-film<sup>16</sup> apparently occurs at each region immediately after the passage of the activation-wave. If this conception is true, the general conditions in living protoplasm resemble those existing in the passive iron model in strong  $\text{HNO}_3$ , where as the activation-wave passes each region there is a chemical and physical disintegration of the surface-film followed by its reformation. This surface change is accompanied by a variation of potential, and hence by the formation of a local current which determines the transmission in the manner already indicated. The evidence that stimulation is associated with a reversible increase in the permeability of the limiting protoplasmic membranes has been reviewed in several of my former papers on stimulation (27), (56), (70). A striking instance where the transmission of the activating effect is clearly accompanied by a rapidly reversed breakdown or loss of continuity in the protoplasmic surface-layer has recently been observed by Just (36) in the sand-dollar egg (*Echinarachnius*) during insemination.<sup>17</sup> At the point of sperm-entry a characteristic local process, associated with the separation of the fertilization-membrane, is initiated in the surface-layer or "cortical zone" of the egg. This change travels slowly over the cell-surface (diameter = *ca.*  $140\mu$ ) and reaches the opposite pole some 20 seconds later. As it travels the protoplasmic surface-film loses temporarily its normal coherence or tenacity, so that if the egg is then placed in dilute

<sup>15</sup> Many striking instances are described in the recent book of Crile (68) on shock and exhaustion.

<sup>16</sup> Of course the degree to which this regeneration involves complete replacement, or simple repair of an altered though permanent structure, cannot be said at present. In the passive iron model an entirely new layer is apparently formed.

<sup>17</sup> Certain details, soon to be published, are included in the following description, with the kind permission of Doctor Just.

sea-water (*e.g.*, 60 vols. fresh *plus* 40 sea) it rapidly swells and bursts, the disruption beginning at the region to which the alteration-wave has extended. This unstable and non-coherent state of the plasma-membrane lasts at each region for only a few seconds, and within about one minute after insemination the whole egg has recovered its normal resistance; it then simply swells without bursting in sea-water of this dilution. These appearances indicate that as the change passes over the egg-surface the external layer of protoplasm undergoes a local disintegrative alteration, losing coherence and semi-permeability; at the altered region a thin film of surface-material is lifted off as the fertilization-membrane; following this process a new coherent and semi-permeable surface-layer is rapidly reformed. In its general features this process resembles that accompanying cytoplasmic cleavage in this egg and in the *Arbacia* egg, where during the formation of the cleavage-furrow the plasma membrane changes its properties in such a manner that the eggs break down rapidly in sea-water of the above dilution; this medium has no such effect in the intervals between cleavage (71). Such a reversible surface-change is probably closely similar to that accompanying the transmission of an activation-wave along an irritable element like a nerve-fiber, with the difference that the processes of local breakdown and reconstruction are much more gradual.

In most of the foregoing examples of protoplasmic transmission the reaction determining the propagation is apparently confined to the surface-film bounding the cell or cell-element from the external medium. Similar transmissions, however, undoubtedly occur within the interior of single cells; and recently an experimental study of these intracellular conduction processes has been made with the microdissection apparatus by Taylor (64), using the ciliated protozoön, *Euplotes*. In this form there are present certain definite intracellular non-contractile protoplasmic strands or fibrils<sup>18</sup> which are connected both with the external motor organs (*cirri* and *membranelles*) and with an internal central structure, the "*motorium*," to which an integrative or coördinating rôle is ascribed. Cutting these various tracts was found to produce definite disturbances of coördination and conduction. It thus appears probable that within the limits of single cells systems of intracellular tracts or filaments may exist, transmitting influence from region to region. The intracellular fibrils of nerve-cells belong probably in this category. If such filaments are bounded by films similar in properties

<sup>18</sup> These strands are similar to those first described by Engelmann (72) in *Stylonychia* in 1880, and to which he attributed a conductive function.

to those bounding the general cell-surface and continuous with the latter, excitatory or similar influences originating at the cell-surface may be thus transmitted through the cell-interior and there modify chemical or other processes. Such a conception would regard the internal protoplasm of many irritable cells (muscle-cells, gland-cells, photogenic cells, etc.) as pervaded by a system of film-structure similar in properties to that enclosing the entire cell; both the structural and physiological characters of the protoplasmic system would then vary with the physical and chemical state of these films.<sup>19</sup> Thus any irreversible breakdown of this film-system would lead to structural and chemical disintegration, as in the cases cited above; but a temporary and reversible alteration, similar to that assumed to occur in the surface-film of a conducting neurofibril, would have a temporary and reversible effect which would cease when the film-structure was reformed.

It appears probable that in contractile fibrillar forms of protoplasm, *e.g.*, muscle-cells, cilia, membranelles, swimming plates, processes of this type form an essential condition of contraction. Removal or alteration of the interfacial film between fibril and sarcoplasm would alter the surface-tension and produce mechanical effects in a manner similar to that seen during the removal of the interfacial film in the  $\text{Hg} - \text{H}_2\text{O}_2$  system (29). It is clear that in a stimulated muscle cell the effect of an alteration originating at the cell-surface is propagated through the entire protoplasm and causes contraction in all of the fibrillar. That the contractile process in the ctenophore swimming plate is associated with a temporary breakdown of the interfibrillar material is indicated by the effects described above as occurring in pure NaCl solutions, especially the loss of coherence between the fused cilia forming the plate; many of these vibrate independently during the brief period of excessive activity, at the same time undergoing a coagulative alteration. The whole appearance is strongly suggestive of a rapid and irreplaceable breakdown of some interstitial material or film-structure which is essential to the normal properties of the whole plate and forms the medium of the coördinated movement of its separate fibrils. This conception corresponds essentially to that of a continuous film-system representing the conductile part of the protoplasm, normal conduction depending on its local breakdown followed by its prompt reconstruction.

<sup>19</sup> Compare Hofmeister's conception (73) of a chambered or film-partitioned structure (related to emulsion structure) as controlling chemical reactions in cells. For example, the rapid onset of autolysis at death may be due to the removal of a film-structure which normally limits the interaction of the intracellular enzymes and their substrates.

The temporary interruption of such intracellular films would permit chemical interaction between compounds which in the normal resting state of the protoplasm are kept apart; and in fact many instances are known where reactions which occur slowly or imperceptibly in the intact cell proceed rapidly when protoplasmic structure is destroyed, or after natural death; the oxidase reaction which causes browning in fruits, potatoes and leaves, and the reactions of autolysis are examples. The recent investigations of Harvey (74) on light-production in animals are especially interesting because of the indirect light which they throw on the inner mechanism of stimulation processes. The substances luciferin and luciferase, whose union in the presence of oxygen determines light-production, are apparently both present in the irritable photogenic cell, but in many organisms their interaction occurs only as the result of stimulation. Evidently some physical barrier preventing this union is then temporarily removed. The sensitivity of a luminous unicellular organism like *Noctiluca* to mechanical or electrical stimulation is most readily understood on the assumption that an intracellular film-structure, which is broken down and replaced in stimulation, controls the photogenic reaction. Light-production thus forms an index of stimulation in the same sense as the bio-electric variation, both processes depending on alterations in the protoplasmic film-structure.<sup>20</sup> The phenomena of irritable gland cells, which show both increased permeability and bio-electric variations during stimulation, are further examples having a similar significance.

The evidence is conclusive that in the surface-films covering passive iron in  $\text{HNO}_3$  or mercury in  $\text{H}_2\text{O}_2$  the progress of the wave of disintegration is the direct result of electrolysis by local circuits. The evidence that transmission in protoplasmic systems is also a consequence of the local electrical currents produced in excitation is mainly of an indirect nature. The above parallels between the processes of activation and transmission in the metallic models and in living protoplasm are, however, of a kind otherwise difficult to explain. The presence of thin alterable interfacial films seems to be the only significant structural feature possessed in common by both systems. Such considerations lend a new significance to the familiar fact that the physiological effects produced by external electric currents, *e.g.*, in muscle, are indistinguishable

<sup>20</sup> An interesting instance of transmission of a light-producing reaction in the colonial cœlenturate animal *Renilla* has recently been described by Parker (75). Waves of light travel over the surface of the organism from the point of stimulation. The  $Q_{10}$  of this transmission is *ca.* 2.

from those produced by normal innervation. On the present interpretation normal innervation represents in reality a form of electrical excitation. It has long been known that the passage of an activation-wave in nerve or muscle is associated with a local electrical variation and circuit (76); and in any electrically sensitive film-partitioned system of the type of living protoplasm such currents must have chemical efforts wherever they traverse the phase-boundaries.

It is clear from inspection (see fig. 3) that the local bio-electric current accompanying the activation-wave, *e.g.*, in a nerve, passes in such a direction that its normal effect—if it causes stimulation under the

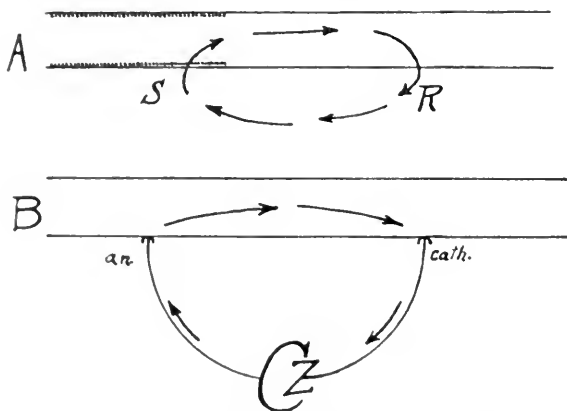


Fig. 3. In *A* the arrows represent the direction of the bio-electric current in the local active-inactive (or action current) circuit on one side of the stimulated or active region *S* (dotted lines). In *B* the course of an external stimulating current from a battery is represented. Stimulation originates, on make, at the cathodal electrode, where the current has the same direction, relatively to the protoplasmic surface, as at *R*.

same conditions as other electric currents—would be to excite the resting areas adjoining the active area, and repress excitation in the active area. Also we know that the action-currents of a tissue have the intensity, duration and rate of change required to excite that tissue (“rheoscopic frog” experiments, etc.). These phenomena of secondary electric stimulation are apparently shown in isolated cells like spermatozoa (77) and ciliated cells, which beat synchronously when in close contact with one another. The synchronous vibration of flagella in protozoa is undoubtedly a closely related phenomenon. The view that transmission of excitation is essentially a case of secondary stimulation by

bio-electric currents was in fact long ago entertained by du Bois-Reymond (78), Hermann (79), Kühne (80), and other earlier students of the bio-electric phenomena. Du Bois-Reymond even speaks of the possibility that processes of electrolysis may underlie electrical stimulation (81).

Many other facts indicate the essentially electrical nature of the factors determining transmission. Thus it is well known that both local stimulation and transmission may be facilitated or hindered by the passage of constant currents through the tissue; this effect is definitely polar (electrotonus), and probably represents in large part an interference effect between opposed electrical currents (37, pp. 136-138). The effects of local injury in blocking nerve transmission may belong in the same category, the local injury current compensating the current of the normal bio-electric circuit as the activation-wave nears the region of injury. Further evidence that the local action-current forms the essential condition for transmission is seen in the effects of altering the electrical conductivity of the medium. Mayor found that in dilute sea-water the rate of transmission in the nerve-net of the medusa *Cassiopea* is less than the rate in normal sea-water, the decrease for a given dilution running closely parallel with the decrease in the electrical conductivity (82). The relation between the conductivity of the medium and the rate of transmission of the contraction-wave in muscle has recently been made the subject of a special investigation by Pond (83), using the *Limulus* heart and vertebrate skeletal and cardiac muscle. The normal medium (sea-water, Ringer's solution) was diluted with isotonic sugar solution, and within a wide range of dilutions a close correlation between the electrical conductivity of the altered medium and the rate of transmission was found. This correlation is to be expected if electric currents traversing the extra-protoplasmic medium are in fact an essential factor in transmission.<sup>21</sup> The distance (from the already active area) at which the bio-electric current traversing the resting area has an intensity sufficient for stimulation must decrease (other conditions being equal) as the conductivity

<sup>21</sup> The fact that stretching a nerve (within moderate limits) leaves its rate of transmission unaltered (96), (97) probably has a similar significance. Excitation is always being initiated in the resting region of the nerve by the current of the local active-resting circuit up to a certain distance in advance of the already active area; on the present theory this is the distance at which the strength of the current where it traverses the surface-film just reaches threshold value, and is determined by the electric resistance, *i.e.*, the *length*, of a column of solution extending beyond the active area for the distance in question.

of the local circuit is decreased; this implies a corresponding decrease in propagation-velocity. One reason why the presence of salts in the tissue media of higher animals is essential is probably that they furnish the electrical conductivity without which rapid transmission would be impossible. The further fact, already cited, that the speed of propagation exhibits a close correlation with the rate at which the local variation of potential rises to its maximum, also indicates that propagation is determined by the current of the local active-inactive circuit. The more rapidly this current comes into existence, the more rapid must be its secondary stimulating effect in adjoining areas and the more rapid the consequent transmission (28).

One further fact of much theoretical interest is that the phenomenon of transmission with decrement, briefly described above for nerve, is exhibited in essentially the same form in the passive iron model as in the living tissue (37). This type of transmission is shown immediately after the wire has undergone complete activation in strong  $\text{HNO}_3$  (*e.g.*  $> 1.25$ ) and has reverted to the passive state; a certain time is then required for the recovery of complete transmissivity; at first an activation-wave initiated at any point travels slowly and for only a limited distance along the wire; by degrees both the rate and distance of transmission increase, and eventually—*e.g.*, in 75 per cent  $\text{HNO}_3$  (*sp. gr.* 1.42) after about 10 minutes (at  $20^\circ$ )—the wire transmits for an indefinite distance as before. Evidently the condition of the newly formed passivating surface-film is such that it is less readily and completely altered or removed by the current of the local circuit than a film which has been exposed to the acid for some time. If the analogy with protoplasmic transmission applies also to the conditions determining transmission with decrement, it is to be inferred that the action of any agent, *e.g.*, an anesthetic, which converts unlimited transmission in a living system (such as nerve) into transmission with decrement, depends upon its altering the properties of the protoplasmic surface-film in some definite manner. On the theory that the normal properties of the irritable living system, especially the normal susceptibility to electrical stimulation and the dependent property of transmitting excitation-waves, are determined by the properties of the surface-films, we should expect that when by any means these structures are rendered less alterable than before, *i.e.*, stabilized, by the action of some physical or chemical agent, activation-waves will fail to be transmitted freely and the system as a whole will then exhibit itself as non-irritable. Reversible effects of this kind apparently form the basis of anes-

thesia, narcosis, and similar reversible inhibitions or suspensions of irritability.<sup>22</sup>

Elsewhere I have attempted a more detailed comparison between the phenomena of activation, transmission and recovery in the living system and in the passive iron model (37). To the electrochemist the possibility of such a comparison may seem doubtful, because in all artificial systems hitherto constructed in which electric currents are produced by chemical action—and in which conversely chemical action is produced by the passage of a current—an essential condition appears to be the combination of both metallic and electrolytic conductors, so arranged as to form a complete circuit. Chemical action is confined to the regions where electricity passes between the one type of conductor and the other—*i.e.*, to the layer of molecules at the boundary-surface. Living protoplasm presents conditions similar to these in some respects and widely different in others. No metallic phase is present; the essential arrangement appears to be a combination of electrolytic conductors partitioned by thin semi-permeable films consisting essentially of chemically alterable colloidal material. Yet chemical reactions are produced in this system by the passage of electric currents; and in its normal activity the system gives rise to electric currents. The bio-electric potentials are small (*ca.* 0.05 to 0.1 volt) in the case of single cells, but they may be summed when the elements are superposed, as exemplified especially in the electric fishes.<sup>23</sup>

If in reality the conditions determining the electrical behavior of the living and the non-living systems are fundamentally of the same kind, it would appear necessary to assume that the semi-permeable films of living protoplasm behave in some way as conductors of the first class. There seems to be no theoretic impossibility in this, and investigation of the electrical conductivity of the water-insoluble compounds present in these films (*e.g.*, lipoids, certain proteins, Ca-soaps) might yield important results, although it is improbable that such substances have more than the low conductivity usual with organic compounds of this

<sup>22</sup> The evidence that these conditions depend primarily upon alteration of the protoplasmic surface-films or plasma-membranes is summarized in my review, *The Theory of Anaesthesia* (84).

<sup>23</sup> For summation of bio-electric potentials *cf.* Brünings (85). The essential structural peculiarity of the electric organ is the arrangement of the elements (plates, discs) in series, with the innervated and non-innervated surfaces of successive elements adjoining each other. This seems to be a clear indication of the electrode-like properties of the surface-layers. *Cf.* Bernstein (86) for a fuller account and literature; also Gotch (87), Biedermann (88), Garten (89a).

type (oils, rubber, etc.). What is of chief physiological interest is the evidence that the primary effects of electrical stimulation are at the protoplasmic boundary-surfaces; apparently it is because of the chemical effects there produced by the current that the surface-films undergo alteration (of permeability, electrical polarization, physical consistency, etc.) during stimulation and transmit waves of chemical change to a distance. The above difficulty largely disappears if we assume that the distinction between conduction in a mass of metal and conduction across a partition of "non-conducting" material loses its importance when the material is in a sufficiently thin layer. Such an assumption is not merely arbitrary, since it is probable that all substances, even water, have some degree of "metallic" conductivity; hence conduction through a layer of oil only a few molecules in thickness need meet with no more resistance than conduction through a thick layer of carbon, metal, or other conductors of the first class. The conditions at the contact of such a thin film of "non-conductor" with an electrolyte solution during the passage of a current between the two would then be essentially the same as at the surface of a metallic electrode. What is essential is that a current should pass across the boundary between the two conductors.

In electrolysis the chemical reaction is confined to the layer of molecules at the *boundary*, where electrons are transferred between the phases. As regards the electrochemical processes occurring at the boundary region it is a matter of indifference whether conduction in the *interior* of either phase is metallic or electrolytic; this can readily be seen if we imagine the metallic phase reduced to the thickness of a few molecules; only the surface molecules are concerned in the chemical process in any case. Adsorption films of oil or similar material need be only one or two molecules thick (89); and in living matter adsorption is undoubtedly one of the chief preliminaries to chemical reaction (90). To explain the behavior of living matter with reference to the electric current, therefore, it seems only necessary to assume that the chemically reactive material is present as an adsorbed layer of molecular thickness at the surface of a thin continuous, water-insoluble film, having a conducting aqueous phase (salt solution) on either side. The passage of a current across such a film would be attended with chemical alteration, and, conversely, chemical alteration (*e.g.*, oxidation of the film material) would yield a current. The plasma membrane, together with the internal protoplasm and external medium adjoining, apparently represents an arrangement of this type.

On the other hand, if the living system, in its current-producing and electrosensitive capacity, represents a special type of electric cell as yet unknown to electrochemistry, we ought to be able to synthesize such cells artificially, instead of depending on the processes of organic growth to synthesize them for us. It seems difficult to believe, in view of the detailed nature of the above parallels, that the living and the non-living systems are fundamentally dissimilar in the conditions determining the relations between electric circuits and chemical change. There is in fact much evidence that under certain conditions the passage of electric currents across inorganic semi-permeable membranes (precipitation-membranes) is attended with chemical change (91).<sup>24</sup> The related facts of electrostenolysis (92) also show that metallic conductors are not necessary for the production of electrochemical effects. The more complete determination of the conditions underlying the electrical sensitivity and current-producing capacity of living matter must, however, await further investigation.<sup>25</sup>

<sup>24</sup> An apparently similar effect is seen at the nuclear membrane of blood corpuscles in the indophenol oxidation reaction; the granules of oxidation-product are deposited most rapidly at the nuclear surface, and their formation is accelerated by induction-shocks (98).

<sup>25</sup> A complete discussion of this problem is not possible here. Its essentials may be defined briefly as follows. The surface of the living cell and the surface of a metallic electrode have certain conditions in common, such that the passage of an electric current between either and the adjoining electrolyte solution is attended with chemical change in the substances at the interface. In the metal-electrolyte combination the carriers of electricity (electrons and ions respectively) are different in the two phases, and each phase is impermeable to the carriers of the other phase. But with a sufficiently steep fall of potential across the boundary and a complete circuit (not compensated), electrons are transferred between the metal and the ions or molecules in contact; in other words, a current flows between solution and metal, and the molecules or ions which lose or gain electrons undergo corresponding chemical change. The chemical rearrangement may involve either decomposition or synthesis, oxidation or reduction, of particular compounds, according to circumstances. In the living cell also the surface is impermeable to the carriers, *i.e.*, a semi-permeable film is interposed between medium and internal protoplasm; this condition allows a P.D. to exist across the cell-surface. The film between the two electrolyte solutions (intra- and extra-cellular) is thin and the fall of potential across the cell-surface correspondingly steep; hence when a current passes the conditions are not essentially different from those at the metallic surface; *i.e.*, there is transfer of electrons between molecules and ions at the interface, with associated chemical change. If this reasoning is correct, we may conclude that the transition from a metallic to an electrolytic conductor furnishes the same conditions (as regards the effects produced by passing a current) as the transition from one solution to another across a thin semi-permeable membrane.

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## ON THE ORIGIN OF THE CELLS OF THE BLOOD

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The history of the development of our knowledge of the blood centers around two opposing theories, and nowhere in medicine can there be made a more interesting analysis of the value of constructive theories, whether right or wrong, in the advance of science. No fact is better known than that the foundation of our knowledge of blood was made by Ehrlich. His introduction of the method of a chemical analysis of cells by means of dyes made modern hematology possible. Ehrlich divided the white cells of the blood into the granular or leucocytic forms and the non-granular or lymphocytic types.

In the leucocyte Ehrlich found five specific types of granulations, only three of which occur in human blood: first, the  $\alpha$  or oxyphilic, eosinophilic granule, which is large, refractive, uniform in size, round as in human blood, or in long rods as in birds; second, the  $\beta$  or amphophilic granule having the power to bind a chromophilic radicle from either a basophilic or an acidophilic dye. This type of granule is found in young cells, never in the adult forms. Third, the  $\gamma$  granulation which is the metachromatic, basophilic granule of the mast cell; fourth, the  $\delta$  granule which is truly basophilic and is not found in human blood, and fifth, the  $\epsilon$  or neutrophilic granulation. This is the fine granulation which in human blood is neutrophilic, showing best in the Ehrlich triacid mixture, and in May-Giemsa stain. In some animals the fine granulation is faintly eosinophilic instead of neutrophilic and is termed pseudo-eosinophilic.

Ehrlich saw in the affinity of these different granules for specific types of dyes an expression of a true chemical reaction and an indication of the division of the cells into specific functional groups.

Since Ehrlich's work it has been shown that eosinophilic granules have a lipoidal envelope which stains in certain basophilic, vital dyes. In 1902 Michaelis and Wolff (41) discovered that there is another type of granule, stainable in azur which is to be found in lymphocytes. In both of the great modern atlases of blood, namely Pappenheim (55) (56) and Ferrata (20), only certain lymphocytes are shown with granules,

and yet I think that granules can be made out in all living lymphocytes though often not more than two or three to a cell. This granule is metachromatic and Michaelis and Wolff thought that this granule was characteristic of the large mononuclear forms and the transitionals as well, but Naegeli (51), (52) showed that the large mononuclear cells and the transitionals belonged together in one group, the monocytes, in having a granule which is not identical with the azur granule of the lymphocyte. At first (1909) he thought that the granule of the monocytes was neutrophilic allying the cells with the myeloid forms, but later (1912) he found that their granule is neither azurophilic nor neutrophilic. It differs from the azur granule chemically because it gives the oxydase reaction while the granule of the lymphocyte does not. The oxydase reaction was introduced by Winkler (69), and consists in producing idophenol blue in cells that have a specific ferment to synthesize the dye from alphanaphthol and dimethylparaphenyldiamin. This ferment is in the granules of the leucocytes and monocytes but not in the lymphocytes.

Ehrlich's classification then led to six types of white cells, the neutrophilic, eosinophilic and basophilic leucocytes, the small lymphocytes with which were included certain intermediate forms, the large lymphocytes or mononuclear forms and the transitionals. The studies on the blood since the time of Ehrlich have led up to a classification of the white cells into three groups; first the leucocytes with their three different cells; second the monocytes, with two different types, large mononuclear forms and the so-called transitional cell and third, lymphocytes. By this classification I mean to indicate specifically the absence of any transitions between the cells of the three main groups, leucocytes, monocytes and lymphocytes.

In the studies of blood and bone marrow made by Ehrlich and following his work, three problems dominated. First, the question of how primitive nucleated red cells became the non-nucleated red blood corpuscles, through tracing the young erythroblasts with large nuclei having comparatively little chromatin, through the stages of the normoblast with a smaller nucleus which becomes gradually pyknotic to the stage of the extrusion of the nucleus. Secondly, the study of how the various types of myelocytes in the bone marrow became the three types of leucocytes by the gradual change of their oval nuclei into the characteristic polymorphic types. Thirdly, the question of the origin of blood platelets. In connection with platelets it is now generally recognized that Wright has demonstrated that they are fragmented parts of the cytoplasm of the megakaryocytes of the bone marrow.

The work of Ehrlich on anemias and leukemias led him to separate the two great groups of blood-forming organs of the adult, namely, the bone marrow and the lymph glands; the bone marrow giving rise to erythrocytes and the myelocytes as forerunners of the leucocytes while the lymph glands give rise to lymphocytic forms. In this system the spleen gives lymphocytes normally but readily returns to its embryonic myeloid function as a compensatory reaction in disease. This is the so-called dualistic, or more strictly polyphyletic theory of the origin of blood associated with the name of Ehrlich, and later with Naegeli, Schridde, Morawitz and others. This theory postulates that there are different stem cells for the reds, for the leucocytes and the lymphocytes and that they are located in specific places in the adult.

It is, I think, exceedingly interesting that though it may well prove that the Ehrlich doctrine is nearer to the truth than the more modern, monophyletic theory, it cannot, I believe, be questioned that most of the progress in the subject of the blood since Ehrlich has been made under the monophyletic theory. A list of the chief names associated with the theory will prove this: Dominici, Pappenheim, Weidenreich, Maximow, Danckhoff, Ferrata.

The foundation of the monophyletic school was laid in the discovery that there can be found for each type of cell in the blood—the erythrocyte, as well as for every type of white cell—a primitive cell with basophilic, azurophilic cytoplasm. Thus we pass into a period in which the study of cytoplasm was stressed more than the nuclei. The identification of this primitive cell as a common stem cell or hypothetical hematoblast is then the standpoint of the monophyletic theory. The first clear account of such a primitive, basophilic cell as a general stem cell is to be found in a paper of Pappenheim (53), in which he calls attention to the fact that Ehrlich had noted that young myelocytes had a basophilic cytoplasm while the only stainable substance of the cytoplasm of their descendants, the leucocytes, was the specific granulation. Pappenheim discovered a primitive stem cell with basophilic cytoplasm as a forerunner of erythroblasts and identified this primitive cell as a large lymphocyte. The working out of this theory has depended on the development of a new series of stains for blood, all modifications of the Romanowski eosin-methylene blue stain, namely, the entire group of azur dyes, the Giemsa stain and all its modifications, May-Giemsa, May-Grünwald, Wright, Jenner, Wilson, etc. Azur is an oxidation product of methylene blue. Azur II, which is the basis of these stains is a mixture of methylene blue and azur. The clearest account of these

methods is to be found in Naegeli's book, *Blutkrankheiten und Blutdiagnostic*.

The best account of the embryology of the blood, from the standpoint of the monophyletic school, is to be found in the work of Danchakoff (6) for birds and Maximow (35), (36) for mammals. Both use the eosin-azur stain after fixation in zenker-formol, and section the embryos in celloidin. Madame Danchakoff finds that the original blood cell is a "primitive stem cell" derived from the blood islands which is neither to be identified as a red nor as a white cell. This primitive cell develops first within the vessels and subsequently extra-vascularly and in part from endothelium. At the stage of eighteen somites in the chick part of these primitive cells have become erythroblasts so that she finds in the vessels two types of cells, namely, the primitive lymphocytes and the erythroblasts. On the end of the fourth day of incubation the granulocytes differentiate from an extra-vascular stem cell, so that the stem cell within the vessels gives erythroblasts, while the stem cell without the vessels gives the granulocytes. The essential conclusion of Madame Danchakoff's work rests on these facts, that she has discovered that the red cells of the chick arise within the vessels, while the granulocytes arise outside the vessels. The cells from which these two groups of blood cells come, look alike and, being regarded as identical, she concludes that a common stem cell gives one or the other group according to its environment. Her four plates give beautiful figures of all of the stages in the differentiation of erythrocytes, from the first appearance of hemoglobin through the stages of polychromasia, which is the name to express that in the eosin-azur dyes basophilic cytoplasm stains blue and hemoglobin stains red and both show at the same time in the same cell.

Maximow (35), (36) has given the best and most complete account of developing blood, using mammalian forms. He finds that the first blood cells of mammals develop within the vessels of the embryonic membranes as do those of the chick; that the primary cells are first lymphocytes, then erythroblasts. He notes the very close relationship of endothelium to the blood cells but thinks that the endothelium gives rise only to indifferent stem cells. Subsequently the formation of blood goes on within the embryo and in the mammal he thinks becomes entirely extra-vascular. Thus he thinks that there are two groups of erythrocytes, the primitive intra-vascular forms that disappear, and the definitive extra-vascular group that is permanent.

He finds that the formation of blood within the embryo is at first diffuse, the cells differentiating from the mesenchyme in many places,

notably in the region of the head; subsequently becoming localized in the liver, in the spleen, and finally in the bone marrow. Maximow believes in a large lymphocyte as a common stem for all the blood cells. He finds that the marked difference between the chick and the mammal lies in the extra-vascular origin of the red cells in the latter, in all but the earliest stages. The extra-vascular origin of the red cells in mammalian bone marrow is now regarded as proved and is generally accepted. It rests on this work of Maximow and on the studies of Mollier (43) on the development of blood in the liver. In connection with the work of Mollier it can be shown from his own figures that the extra-vascular spaces he describes may just as well be interpreted as a part of the lumen of the developing vessels in the light of our present knowledge of the differentiation of blood vessels from a vasoformative cell, the angioblast. In connection with the origin of red cells in bone marrow, we have been up until now without complete knowledge of the anatomy of the vascular system in that organ. It has been shown in some work by C. A. Doan, in this laboratory, soon to be published, that there is a very extensive plexus of collapsed capillaries in the adult bone marrow of the pigeon. Thus the subject of the origin of red cells in bone marrow must now be re-studied in the light of this better understanding of the structure of the organ.

In 1907, Maximow (38) followed the evolution of bone marrow by stimulating the formation of bone and marrow in the kidney by tying off the renal vessels in the adult rabbit. By this experiment he obtained a marrow much more simple than that of normal development and one in which it was easy to follow the stages, which are a preliminary dilatation of the small veins giving a slowing of the circulation and the subsequent differentiation of the various types of cells from a basophilic stem cell. The earliest identifiable red cell he calls a megalo-blast, which has a very large pale nucleus, then an erythroblast and finally an erythrocyte. He gives a careful description of the evolution of each type of cell and an especially clear account of the differentiation of the granulocytes by a gradual increase in the specific granulation in a primitive, basophilic stem cell.

The presentation of the monophyletic theory has been the life work of Pappenheim in Germany and of Ferrata in Italy. In 1905 to 1912 Pappenheim published his well-known atlas, and since Pappenheim's death the *Folia Haematologia* (56) has published his final monograph which covers the entire field of hematology.

The presentation of the various forms of the blood cells in the adult and in development as they can be seen in the azur-cosin technique, in

normal and pathological states in these two atlases and the two papers of Maximow and Danchakoff, cannot be surpassed. The monograph of Pappenheim covers completely all the known facts of blood and is a real mine of accurate information but made excessively abstruse by countless genealogies of blood cells which I think are all based upon a misconception of the origin of blood. With the elimination of Pappenheim's hypothetical "lymphoidocyte" all of this complexity would disappear and this complexity could indeed drop out without detracting from his achievement. The work of Pappenheim in founding the monophyletic school and in tracing the maturation of individual blood cells has led and dominated hematology since the work of Ehrlich.

The atlas of Ferrata (20) also covers the whole field of modern hematology. He brings out further stages of the evolution of the specific types of blood cells as a completion of the main contribution of the monophyletic school. His first ten plates cover the maturation of the red blood cells most completely and beautifully. He has worked out the changes in the azurophilic cytoplasm leading up to basophilic punctation in experimental lead poisoning. The most important section of his book is the one in which he deals with the relation of the blood cells to the connective tissues in general, a subject of great interest, as will appear later.

To sum up the work of the monophyletic school, they have shown that the original cell of each series of the blood cells has a basophilic cytoplasm staining especially in azur; they have given us a complete and accurate history of each specific cell; they have shown that the erythrocyte begins as a cell with basophilic cytoplasm in which the acidophilic hemoglobin gradually appears so that the young cells have a stage when both substances stain, called polychromasia, which is gradually succeeded by the ripe forms in which the only stainable substance is the acidophilic hemoglobin. They have described more fully the stages in which granulocytes begin by a development of specific granulation in a primitive basophilic cytoplasm and finally in their beautiful atlases of lithographic plates have illustrated the entire range of variation of the three types of white cells, namely the lymphocytes, the granulocytes (leucocytes) and the monocytes. The lymphocytes include all the small lymphocytes and the older cells up to those about twice the size of the small cell. The monocytes include the very large, pale, mononuclear forms, and all of the transitional forms of Ehrlich. The actual separation of monocytes (histiocytes) as a specific functional group is due to Aschoff and Kiyono. The monophyletic

school have, I believe, made a mistake in the identification of the lymphocyte or a lymphocyte-like form, as a common stem cell. It is, I think, unquestionably true that the primitive stem cells of the different groups of blood-cells cannot be separated in fixed specimens even with the most perfect technique, but I think that these stems can be analyzed with the method of vital staining.

Naegeli has been the leader of the polyphyletic school since the time of Ehrlich, and I think that it is fair to say that in spite of the brilliance and the dominance of the monophyletic school, the idea first indicated by Naegeli of classifying the white cells of the blood into three groups, leucocytes, monocytes and lymphocytes, has been used continuously in the clinical study of blood, the reason for which now being clear, that this classification is functional.

*Clasmatocytes.* We are at the present time in the beginning of the experimental phase of hematology. As soon as we pass into this phase it becomes clear that blood, both embryologically and functionally, must be considered as a part of the larger subject of the connective tissues. The beginning of the experimental analysis of the connective tissues was made by the Russian, Maximow, one of the greatest of modern histologists. The first step was in some experiments on rabbits published in 1901-02 in which he introduced two sterile cover-slips under the skin in rabbits and found that leucocytes were the first cells to wander between them; then followed a specific type of cell which he called a clasmatocyte and for nineteen hours only these two cells were present and it was not until later that typical fibroblasts appeared. Thus he showed that in the main there are two distinct strains of cells in the subcutaneous tissue, clasmatocytes and fibroblasts. Four years later he defined the clasmatocyte more specifically by the use of a vital dye, neutral red. If living connective tissue be stained with 1 per cent neutral red, all the cells will be killed and all will stain, but if the dye is used in the strength of 1 to 10,000, and injected into the blood vessels, only the clasmatocytes will react and thus they stand out most clearly. The clasmatocyte is a cell with an oval nucleus that is smaller and stains more intensely than that of a fibroblast. The cell varies in shape, is oval or irregular and has more definite contour than a fibroblast. Its cytoplasm contains granules that stain with neutral red, and mitochondria, and its most distinctive characteristic is certain vacuoles, which are really fluid parts of the cytoplasm which also stain with neutral red.

The next step in our knowledge of the clasmatocyte came in the work of Bouffard (4) in 1906 and was a by-product, as it were, of experiments

in connection with the treatment of trypanosomiasis by the injection of benzidine dyes. Ehrlich had injected trypan red hypodermically and had noted that the animals became stained. Following this work and that of Mesnil, Bouffard injected "Toluidin and acid H," and found that the dye was taken into the blood-stream from which it quickly disappeared by being taken up by the Kupffer cells of the liver, the cells of the renal tubules and by certain interstitial cells (clasmatoocytes) of organs. Three years later Goldmann (22), directly through the influence of Ehrlich, took up the method and really established it as a method for studying phagocytosis. He first used pyrrol blue and later the less toxic trypan blue and gave a very searching analysis of the reactions of the various types of the cells of the body to these dyes. He brought out sharply that there is a specific cell of the connective tissues, which he called the pyrrol cell, that can be clearly separated from other cells by this experiment.

The method was then used by Evans and Schulemann (15), (16), (17) and a very large group of workers, with the result that the clasmatoocyte can now be very accurately defined. Evans and Schulemann proved that the azo dyes do not stain any part of the cytoplasm but that the dye is taken into the cell as particulate matter of specific size and that these granules are segregated and clumped in vacuoles. Neutral red, used as a vital dye, that is, in specific dilutions, stains both preformed granules in these cells and the fluid in the vacuoles. These vacuoles are an organ of the cell for segregating particulate matter which has been called the segregation apparatus by Evans who gives all their synonyms, plasmasomes by Ferrata, the grains de ségrégation by Renaut, vacuoles à grains de ségrégation by Dubrueil and purpur granula by Hammer, dye sphere formations by Rosin and Bibergiel, and more simply the vacuoles by Lewis and Lewis.

In an extensive monograph of Evans and Scott (18), which is now in press in the Contributions to Embryology of the Carnegie Institution of Washington, is given a most complete and beautiful description of the reaction of the clasmatoocytes on the one side and the fibroblasts on the other to about 200 of the known vital azo dyes in both acute and chronic stages. Both clasmatoocytes and fibroblasts take up the dye as minute particles, the clasmatoocytes in greater amount, and the clasmatoocytes have vastly greater power of clumping the particles into larger masses and segregating these masses in vacuoles. There the substance is either retained in amorphous clumps or, as Evans and Scott have shown, may form beautiful crystals. They find that though

in very chronic condition the amount of the dye in fibroblasts may so increase as even to exceed that of the clasmatoocytes and that though fibroblasts develop an extensive segregating apparatus, nevertheless even in these extreme conditions the cells do not become identical and can be separated by the fact that the clasmatoocyte gives up the dye much sooner. This work will live as the standard for the complete differentiation of these two types of cells. Every student of histology and pathology should see the clasmatoocyte after staining *in vivo* with trypan blue and with the supra vital staining of neutral red. In either experiment a bit of the omentum or a little of the connective tissue around the pancreas gives a most beautiful demonstration. The differences between the clasmatoocytes and the fibroblasts are also most clearly brought out in the method of tissue culture, as shown by Lewis and Lewis, for against the delicate films of fibroblasts spread out in one plane in their preparations, the clasmatoocytes show with great clearness.

It is obvious that phagocytosis is a very general property of cells and the original experiments of Goldmann gave a survey of almost all of the types of cells that take up these benzidine dyes which are introduced in the form of particulate matter. The question of the reaction of leucocytes to these dyes has been an interesting one. It is well known that leucocytes are phagocytic, indeed vitally stained with neutral red many of them can be shown to have a stainable vacuole but they are found stained only occasionally after injections of these dyes. Downey (10) showed that they were stained if taken from an occluded vein in which there was dye in the plasma or if taken from the tissues soon after a subcutaneous injection. The question of the reaction of all of the types of the cells of the blood to phagocytosis must be taken up in the light of the fact that it has been conclusively shown that foreign particles remain in the blood stream but a short time. Drinker (11) has recently tested this by using a substance, manganese dioxide, which can be recovered chemically and has followed the fate of a given dose in the body. He has found that in most experiments all of the particles have disappeared from the blood in eighteen minutes, and that at the end of one hour 90 per cent of the material can be recovered from the liver, the spleen and the lungs. The question is therefore one of the relative power of cells in phagocytosis, and there are special cells in these three organs which possess this power in the highest degree. These special cells are all endothelial cells. It is obvious that all of the dye is not permanently stored in these special endothelial cells and

the clasmatoocyte thus stands out as a cell for storing particles over long periods of time. But in the light of these experiments a negative finding in connection with the blood cells does not prove that they cannot take up the dye since in any experiment the dye is in the blood stream so short a time.

The clasmatoocyte has now been most effectively discriminated from the mesothelial or serosal cell lining the pleural and peritoneal cavities in some experimental studies of Cunningham (5 a). This relationship has been the subject of very extensive studies, notably by Pappenheim, Weidenreich and Schott. Cunningham has been able to analyze the reactions of serosal cells to various types of stimuli and has found that their reactions are entirely different from those of the clasmatoocytes, the fundamental physiology of the former being differentiated toward secretion while that of the latter is toward phagocytosis. In fact he has shown that the serosal cell is more like a fibroblast than it is like a clasmatoocyte. The serosal cell may take up particles of dye, it may become free, but it does not become a clasmatoocyte. It is clear that modern histological studies depend on the discovery of such specific criteria for cells as to enable one to distinguish them from all other cells moreover these criteria are often the keys for analyzing their functions. A general survey of cells with reference to these specific criteria is being made in the beautiful studies of living tissues by the method of tissue culture in the work of W. H. and M. R. Lewis.

The differentiation of the clasmatoocyte from all the other types of the cells of the connective tissues is the first step in the newer work in hematology. The clearest presentation of the idea of the relationship of the clasmatoocyte to the monocytes of the blood we owe to Aschoff and Kiyono. They studied the reactions of cells after the injection of lithium carmine into the circulation, in fact, they say that this procedure was first introduced by Heidenhein. In these experiments they found that the white cells which occur in the thoracic duct, that is to say, the lymphocytes, did not take up the granules of the dye but that there was a group of blood cells especially numerous in three places, the veins of the spleen, the vessels of the liver and the veins of the extremities, which did take up the dye exactly as do the clasmatoocytes in the experiments of Bouffard and Goldmann. The fact that not all of the blood cells of one type take up such particles in any one experiment need no longer be a confusing factor in the light of the speed with which such particles are taken out of the circulation. Aschoff and Kiyono regarded these cells as of endothelial origin, and like the clas-

matocyte of Maximow or the adventitial cell of Marchand. They called them histiocytes or endothelial leucocytes. They therefore stressed the division of the white cells of the blood into three types, leucocytes, lymphocytes and histiocytes or monocytes. At the meeting of the American Association of Anatomists in 1921, Simpson described the vital staining of films of human blood by the Pappenheim method in which she showed that the transitional cell of the blood stream has a vacuole which is identical with the segregating apparatus of the clasmatocyte. In some studies on the differentiation of blood cells as seen in the living chick (1921) I have been able to prove that the clasmatocyte of the connective tissues and the monocyte of blood are identical cells with an identical origin. Both of them can be seen to differentiate and become free from the endothelium of capillaries and veins in a living form. Ferrata's view that the clasmatocyte may be considered as a stem cell does not seem to me justified because both cells are completely differentiated when they break off from the parent endothelium.

*The erythroblasts, the first blood cells.* In giving an account of these experiments with a living form one should begin with the erythroblasts since they are the first blood cell to form. Moreover since erythroblasts come from angioblasts and endothelium one must begin with the subject of the origin of the angioblast.

In 1920 the writer (61) published an account of a study of the origin of the vascular system as it can be made out in the living blastoderm of a chick of the second day of incubation. During the second day of incubation the essential processes of the origin of the vascular system can be made out in the area pellucida with very great clearness. Blood vessels start by the differentiation of a new type of cell, the angioblast, from the primitive mesoderm. This cell develops a denser cytoplasm than the primitive mesoderm, due to the fine azur granulation so that it is identical with the stem cell of the monophyletic school. The size of these granules is such as to give the cell the appearance of ground glass. During the second day of incubation all of these basophilic cells can be identified as angioblasts because, as seen in the living form, they remain together after their first division to make syncytial masses. These masses of cells then put out very characteristic sprouts by which they join similar masses to form a plexus. These solid masses of cells become blood vessels by a true cytolysis or liquefaction of the center of the mass to form blood plasma while the cells on the edge differentiate into endothelium. The essential characteristics of angioblasts are the dense, basophilic cytoplasm, the tendency to form syncytial masses,

the sprouting to form plexuses, and the liquefaction to form blood plasma. It should be made very clear that there is a tissue fluid before there are any angioblasts, which in the chick is taken in from the yolk by endodermal cells, and that the blood vessels produce their own fluid so that endothelium from the start is a membrane between two fluids, a tissue fluid and a plasma, and the plasma is made from a solution of cells, the nuclei being involved as well as the cytoplasm. It may thus be said that the fundamental morphology of the vascular system has been established.

The next point of importance in the study of these living chicks is that the origin of red blood cells both from primitive angioblasts and from the cell division of endothelium can be proved beyond question. It can be seen in the living chick that when liquefaction is going on, a part of the solid mass may remain and develop hemoglobin as can be made out by the cells becoming yellow or if the cytoplasm of the center of the mass has entirely liquefied the endothelium may divide and give two rows of cells, the inner one of which protrudes into the lumen and will develop hemoglobin while it is yet attached to the wall and divide to make a mass of red blood cells. Thus by watching the living chick of the second day it can be seen that all the primitive blood cells become erythroblasts and that their genealogy is angioblast directly to erythroblast, or angioblast, endothelium, erythroblast. Thus the postulation of a lymphocyte as a stem cell is here unnecessary.

During the present year I (62) have been making further studies of the development of the blood, and have found that the older chicks can be studied in the same manner, the processes being watched under an oil lens. It has been found to be a very great advantage to study these blastoderms with a vital dye, and neutral red is the most convenient. The embryo is studied in Locke-Lewis solution, to which has been added 2 to 3 drops of a 1 per cent aqueous solution of neutral red, a dilution of approximately 1:10,000. From each blastoderm I first draw out one or two drops of blood for films, which are studied with vital dyes and then stained with Wright's eosin-methylene blue. The blastoderm is then mounted and watched for about four hours. The technique for the blood films with a vital dye is that of Pappenheim, to make a very thin film of the dye, neutral red or brilliant cresyl blue, by drawing a clean rod dipped in a saturated alcoholic solution of the dye across a clean slide, and then mounting a drop of fresh blood on this dry film of stain. The specimen is at once ringed with salvoline and placed in a warm box.

In these studies it has been possible to establish a specific criterion for a primitive red cell. At the stage of the second and third day, of incubation, all of the red cells have a very characteristic massive granulation stainable vitally in neutral red and brilliant cresyl blue. It is in the form of granules and rods which make a dense rosette or wreath that completely surrounds the nucleus. It takes a stronger solution of the dye to bring out this granulation than to stain the granules and vacuoles of the elasmatoocytes. In the weaker solution the granulation of the red cells becomes just visible though it is not stained. In the living cell the granulation has the same index of refraction as the rest of the cytoplasm and is therefore not to be distinguished. It is stainable however in a dilution that does not stain the nucleus. The granulation is soluble in all of the fixatives, in Helly's solution, in alcohol, in formalin and in the vapor of formalin but if it be stained in brilliant cresyl blue it becomes insoluble in methyl alcohol and so the cells can be counterstained. In the early stages of the red cell or megaloblast the granulation is massive and completely fills the cell; then as the cytoplasm increases the granulation remains in a rosette or wreath around the nucleus making the erythroblast; a little later it begins to spread out or thin out in the cytoplasm making extensive reticular forms. These reticular forms or erythroblasts begin to appear on the third day and they show both the basophilic cytoplasm and the specific granulation. By the seventh day the majority of the red cells are in the reticular form, but the primitive stage and the rosettes are still present. The primitive red blood cells are fairly uniform in size, or at least on the second day one can make out but a few different generations in them, but by the fifth day there is a remarkable variation with very small actively dividing cells, many of them irregular or spindle shaped and much larger, older forms. The smaller forms have the granules in the rosette form, the larger one in the diffuse reticular form. In both of these stages there is polychromasia. Gradually the amount of the granulation becomes very much reduced. The intermediate stages between the seventh day of incubation and the time of hatching I have not yet followed through, but at the time of hatching and for the first three days afterward, all of the red cells have a few granules stainable with brilliant cresyl blue. Some of the cells have only one or two, others up to eight or ten granules while a few of the cells have more. In this stage there is no longer any diffuse basophilia.

This substance I believe to have very great significance in the study of blood. It forms a specific criterion for recognizing the primitive red

cell in distinction to other types of the blood cells. The method of embryology gives a chance to follow the stages in its development with exactness, so that one can know how primitive a given cell is by the amount and the arrangement of these granules. Since it is so fundamental, it must necessarily appear in the blood in conditions when blood is regenerating. Besides this, it may offer a chance to study the development of hemoglobin, for example, in testing whether these granules contain iron or not.

The use of vital dyes for the staining of granules in blood cells was suggested by Pappenheim as early as 1894, recommending brilliant cresyl blue at that time, while in 1896 Isreal and Pappenheim (26) showed that if a few grains of neutral red were placed on a slide and used for a blood film, a few granules in the red cells would stain as the cells began to die. The use of neutral red for the blood was introduced by Ehrlich. The subject of vitally stained granules in the red cells was not brought into prominence until 1907, when Cesaris-Demel, Pappenheim and Ferrata all published articles on the subject in the *Folia Haematologica*. Cesaris-Demel describes and illustrates the substance both in the granular and in the filamentous form, showing differences in the reaction to the stain of these two forms. He discusses the occurrence of cells containing these granules in cases of anemias. The question which comes up first in connection with this vitally stainable substance is its relation to the so-called "punctate basophilia." Both Pappenheim and Ferrata agree that the two substances are entirely different. Basophilic punctation, or Grawitz granules, consists of fine particles of basophilic substance, stainable after fixation, in azur, exactly like the finely granular basophilic cytoplasm. Ferrata (20) believes that these granules, basophilic punctation, that is to say, granules staining in azur, arise as an abnormal clumping "conglotation" of the primitive basophilic substance. On his plate VIII (20) he shows the changes in the basophilic substance in experimental lead poisoning, the earlier phase being a clumping of the basophilic substance in the periphery of the cell, giving patterns very different from those of the vitally stainable substance. This view of Ferrata, that the basophilic punctation is an abnormal change in a young cell, explains why this granulation does not appear in the normal development of blood. That is to say, basophilic punctation would then represent a young cell which has suffered abnormal changes, while the reticular cells are normal young cells which may appear in the circulation under abnormal conditions. The granules which stain vitally I believe to

have great significance as being a fundamental substance in evolution of the erythrocyte, which would make clear their appearance in the cells of the circulating blood under conditions of regeneration just as nucleated forms appear in regenerating human blood. When the exact stages of the development of the vitally stainable granules are worked out in the human embryo, as can now be done, it will be possible to estimate just how primitive are the young cells that get into the circulation during the phase of regeneration.

In these studies in the chick four different kinds of stainable substances have been found in the cytoplasm of the erythroblasts. First, the very finely granular, basophilic cytoplasm, staining in azure and appearing like ground glass. This substance is not distinctive because it is characteristic of the primitive type of all blood cells. It disappears as hemoglobin develops. When both substances are stained in the same cell, we have the phenomenon of diffuse basophilia, or polychromasia or polychromatophilia. Secondly, a specific granulation characteristic of the erythroblast, a criterion to separate it from other cells, a substance which is exceedingly soluble but can be made insoluble by combination with certain vital stains, notably neutral red and brilliant cresyl blue. When so stained it is retained in methyl alcohol so that the cells can be counterstained in azur-eosin. It is in the form of granules and rods, which are arranged in characteristic rosettes or wreaths around the nuclei; later the substance becomes less dense and then occurs in a reticular form, and finally, in stages after all of the basophilic cytoplasm has disappeared, is in the form of a few granules or droplets, in the acidophilic hemoglobin-bearing cytoplasm. Thus it persists longer than the basophilic cytoplasm. Thirdly, hemoglobin. In the primitive cell there is the basophilic cytoplasm and the specific granulation. Just the moment of the beginning of hemoglobin is hard to make out, but it can be recognized in a living cell before it can be fixed and stained. It occurs in the cells on the second day of incubation when the specific granulation covers the entire area of the cell. When the hemoglobin can first be stained the azurophilic cytoplasm predominates as has been shown by Pappenheim, Maximow and Danchakoff. The second stage of the red cell has polychromasia and the specific granulation in a dense rosette around the nucleus, the third stage has still polychromasia and a reticulation of the vitally stained granules, and finally the basophilia disappears while the hemoglobin and a remnant of the specific granules are left. This stage characterizes the cells at the time of hatching in the chick.

Fourth, there are the so-called Howell-Jolly bodies. These are fragments of nuclei which were discovered by Howell (25) in 1890 in erythrocytes of the cat after hemorrhage. It is very interesting to note that cells with fragmented nuclei, giving these bodies, occur as early as the second day in the chick and are to be found in almost all specimens. They are to be interpreted as dying cells. They show that there is a certain amount of cell-death even in very early stages of development.

Besides these four substances which are to be found in the course of normal development in the chick, there are two other stainable substances found in the red cells in clinical work which have not been found in the chick. They are the basophilic punctation already discussed and the ring bodies of Cabot, especially well illustrated by Ferrata. The latter are thought to be the occasional remnants of the nuclear membrane, after extrusion of the nucleus. It is clear that an accurate knowledge of the maturation stages of the red cells, or the steps by which a primitive red cell becomes an erythrocyte, is of very great value in estimating the severity of an anemia or in following the stages of recovery. The method of studying the development of the blood by taking it out from a chick on each succeeding day of incubation and using the technique which combines vital dyes with the eosin-azur blood stains enables one to follow the stages step by step as cannot be done in bone marrow. In the chick a specific new stage is added with a given increase in the age of the chick. Thus one can have a specimen of red cells in which there are only the primitive types, and then are added the rosettes or wreaths of the specific granule, then the reticular forms in cells which show polychromasia and finally comes the stage of remnants of the specific granulation in erythrocytes with acidophilic hemoglobin. At the time of hatching all of the red cells in the circulation of the chick show a few of these vitally stainable droplets, some of the cells having 1 or 2, others 8 or 10. The same progression in the development of blood can be followed by this method in mammalian forms, but so far only a beginning has been made.

That endothelium may give rise to red cells was discovered by Dan-chakoff (6) and confirmed by Maximow (35), Minot (42), Emmel (12), (13) and Jordan (27), (28). The extent of the origin of the red cells from endothelium in the chick was not made clear until studies were made in the living form. The significance of the origin of erythroblasts from endothelium in the chick will, I think, be much less if Maximow's view is finally confirmed that the origin of red cells from endothelium is only transitory and that the secondary, permanent

red cells are extra-vascular in origin. The question has two phases, are the cells actually extra-vascular, and if so are they related to endothelium?

*Origin of monocytes and clasmatoocytes.* On the third day the white cells of the blood begin and the origin of two groups can be seen in the area pellucida, namely, the monocytes and the granulocytes (62). By the term monocyte I mean the large mononuclear forms and the transitionals of Ehrlich, grouped together in the third row in Pappenheim's plate 1, (56) and the fourth and fifth rows on Ferrata's plate XII (20).

Of the monocytes, it is the transitional forms that are the easiest to make out because they are identical with the clasmatoocytes. On the third day the endothelium of the capillaries and veins of the area pellucida becomes reduplicated in places. All of the endothelial cells have granules that stain in neutral red, scattered throughout the cell. A single cell of the endothelium of the inner row enlarges, protrudes into the lumen, develops the vacuoles that are characteristic of clasmatoocytes, and may even engulf a red blood cell. This I have seen in the living chick and such a cell is shown in Maximow's figure 4 on plate XVIII (35). The free border of the cell then develops an appearance characteristic of clasmatoocytes, a film of cytoplasm in which a central process is more refractile than the rest, and these films of cytoplasm are in constant motion. These cells I have seen become free from the wall making the transitionals of the blood. Maximow has seen these cells in the vessels of mammalian embryos and has shown three of them in the same figure but did not regard them as a blood-cell type. They are identical with the clasmatoocyte and with the transitional forms of adult blood. At the same time the endothelium gives rise to clasmatoocytes; the outer cells of a reduplicated endothelium divide rapidly and make irregular patches of cells along the vessels which differentiate vacuoles, many of them very large, and these cells become free and have all of the characteristics of clasmatoocytes. The extra-vascular ones tend to be larger than those within the vessels but I have seen one of the large forms pass into a capillary. Thus the transitional cell of the blood and the clasmatoocytes are identical forms and both develop from endothelium. Moreover they become differentiated into the specific cell type before they are detached from the wall of the vessel.

The large mononuclear form comes also from the endothelium though this is less easy to demonstrate on the third day because there are so few and because one lacks the characteristic neutral red reaction to pick them out. A special endothelial cell may enlarge and become free

with neither the neutral red rosette of the erythrocyte nor the vacuoles of the clasmatoocyte. The cells become larger than the others and their nuclei become eccentric. They are most readily demonstrated in Wright's stain after the vital dye brilliant cresyl blue because they lack the characteristic granulation of the red, and because their cytoplasm stains a distinctive clear blue. Thus in the yolk sac of the living chick of the second and third days of incubation it can be proved that endothelium gives rise to the erythroblasts, to the monocyte stem of the white cells and to the clasmatoocytes of the connective tissue. On the second day almost all of the cells become erythroblasts, though I have found a few monocytes within the vessels in an occasional specimen. In other words, it may be said that the clasmatoocytes are from endothelium, most of them are extra-vaseular, a few intra-vaseular.

Having proved that the monocytes of the blood and that the clasmatoocytes of the connective tissue are derived in the embryo from endothelium and are identical cells we may now go a little deeper into the subject of the clasmatoocyte. Maximow distinguished between the resting wandering cell and the active one in his first experimental procedure, indicating that the mononuclear cell of the connective tissues, well known to the pathologist, became transformed by irritation into the actively phagocytic clasmatoocyte. The same double group of cells is to be found among the monocytes of the blood, in the large mononuclear and the transitional forms. Both come from endothelium, and it is a question whether the mononuclear becomes in the blood stream a typical transitional or not; in Pappenheim's row of monocytes (56, plate I), the second cell is an undoubted large mononuclear but has a vacuole in which is a remnant of a phagocytized red cell. The large mononuclears of the blood stream may have the relation to the transitional that Maximow sees in the mononuclear cell of the connective tissues to the clasmatoocyte, namely, a resting and an active phase of the same cell, or the mononuclears of the blood stream may be for the most part old cells, which would not give rise to typical transitionals. Maximow called the whole phagocytic group of cells of the connective tissue, polyblasts, clasmatoocytes, or resting and active wandering cells. He believed that they arose from blood cells (lymphocytes) which had wandered out of the vessels. Marchand (34) then took up the subject and carried it a step farther showing that the clasmatoocyte is not a cell which has emigrated from the blood vessels, but is rather a true extra-vaseular cell, which he called the adventitial cell.

The term *clasmatoocyte* was adopted by Maximow from Ranvier who had given the name to an amphibian cell now known to be different; the word means to fragment and in this sense is not descriptive. It has been called the resting wandering cell and the polyblast by Maximow, the histiocyte by Aschoff-Kiyono, the pyrrol cell by Goldmann, the rhagiocrine cell by Renaut and finally Evans has adopted the term macrophage to include all the cells of the connective tissues that phagocytize trypan blue. It is obvious that the term macrophage is not distinctive for the one type of cell, especially since we can now separate the *clasmatoocyte* so sharply from the serosal cell. Now that this cell-type has become so clear that we know its origin and its specific function, we should have a distinctive and a clear name. We may then offer as a definition of a *clasmatoocyte* or histiocyte, that it is a cell derived from endothelium being very highly specialized toward phagocytosis, and possessing a marked development of vacuoles in which particulate matter is segregated, agglutinated and stored and having a differentiation of the periphery of the cytoplasm into films that keep the cell in motion, while the cell itself has only a slow locomotion.

With this definition in mind it will be interesting to take up the work of Mallory and his school on the "endothelial leucocyte," which brings us to the question as to whether *clasmatoocytes* differentiate in the adult. In 1898 Mallory (33) described the reaction of lymph glands to typhoid infection. He first took up the reaction of the follicle proper and then the reaction of the sinus. First in regard to the reaction of the follicle he says:

In the lesions to be described, however, the large cells of the reticulum of the lymphoid tissue behave in all respects like the endothelial cells of the lymph and blood-vessels. This fact might be used as an argument, therefore, in favor of their being endothelial rather than connective tissue cells. In the following pages these cells will be spoken of as endothelial cells. Under the influence of the toxic product of the typhoid bacilli these larger endothelial cells increase in number.

This indicates that Mallory believed that the reticular cells of the follicle are normally covered with endothelial cells, and he makes this statement still more clearly in his *Pathological Histology* (p. 616). Here it must be said that it is not thought by histologists that in normal lymph glands there is such a spreading of endothelium over the reticular cells of the follicle. The follicle consists of a framework of reticular cells, through which passes a capillary bed; the interstices being filled with lymphocytes. Mall gave us a clear picture of the reticular

cell; it is a branched cell of the connective tissue which produces a specific type of fiber, namely, one which is branched to an extreme degree wherein it differs from the unbranched white fibers, but is nevertheless much more closely allied, perhaps identical with the white fiber chemically. The reticular cell differs from the fibroblast which produces the white fibers and the yellow elastic fibers in being more primitive, namely, in that the reticular fibrils remain within the cytoplasm of the cell throughout life. Since in the normal follicle there are no endothelial cells on the reticulum, it may well be questioned whether the "endothelioid cells" occurring pathologically in the follicles have arisen in situ from reticulum, instead of being clasmatocytes which have wandered in.

The structure of the lymphatic sinus and the splenic pulp does, on the other hand, bring out a much more complicated relation between endothelium and reticulum. While in the follicle there is normally one cell, in the sinus there are two. That cells of the monocyte type become free in the sinuses is without doubt, and the question is, can their origin be analyzed, can it be shown that they are endothelium or reticulum? Embryologically the sinus is a zone where there is a plexus of lymphatic capillaries lined by endothelium with bridges of reticulum between, or as in the spleen, a plexus of vascular capillaries with reticulum. In the development of the sinus, the lymphatic plexus or the vascular capillary plexus has become so extraordinarily dense, that the bridges between adjacent vessels are reduced to single branched reticular cells (59). In other words, endothelium is spread over reticulum. Such being the development of the sinus, the question comes up as to whether these two types of cells, which are present at first, can be discriminated throughout life, and this question was ably discussed by Downey at the meeting of the American Association of Anatomists in 1915. In discussing the sinuses Downey (8) said:

Even with ordinary methods it is evident that the strands of the reticulum are composed of branched, anastomosing cells which are closely associated with the fibers. Nothing can be seen of a continuous epithelial covering. Associated with these strands, especially where the reticulum forms the wall of a sinus, are varying numbers of larger and more rounded protoplasmic cells whose connection with the fibers of the reticulum is not so evident with ordinary methods. Such cells, especially where they project out into the lumen of a sinus, might well be mistaken for hypertrophied endothelial cells. However, the use of any one of the numerous specific stains for reticular fibers (Krause's iodo-iodide of potassium—gold chloride method, the Maresch-Bielschowsky, or the older formula of Mallory's hematoxylin as used by Thomé) shows clearly that these cells are

frequently traversed by fibers, and that even the large rounded cells resembling large mononuclear leucocytes are frequently attached to the reticulum and have fibers embedded in their peripheral portions. These latter cells show great phagocytic activity, especially for red corpuscles, and their nuclei are large and indented. If these cells were not attached we should not hesitate to pronounce them as large mononuclear leucocytes.

Thus Downey recognized both types of cells clearly but regards them as identical on the basis that both of them contain fibrils. In this connection it is interesting to remember that Mall demonstrated that endothelium can produce reticulum, as in the liver, while Corner has shown that this is true also in the corpus luteum of the ovary. Thus it may be questioned as to whether the presence of reticular fibers is to be considered as an adequate separation of endothelium from reticulum. The question is significant in connection with such a specific development of the power of phagocytosis as has been demonstrated as a characteristic of endothelium and its derivatives. It may be accepted that these two cells cannot be distinguished in sections of sinuses by histological methods. The question then comes up as to whether these cells can be separated by experimental methods. All of the experiments with the injections of particulate matter have brought out that there are four special places where certain cells always phagocytize considerable amounts of any injected particles. These places are the capillaries of the liver and the veins of the splenic pulp, in both of which areas the cells are unquestionably endothelium. The other two places are the lymphatic sinuses and the sinuses of splenic pulp and in both of these areas the question is complicated by the relation of endothelium to reticulum. McJunkin (40) has recently made some experiments with the injection of carbon particles in the form of India ink. He analyzed first the nature of the suspension of carbon particles, reducing the size of the particles by filtration. He found that if filtered India ink be mixed with sodium citrate, the polymorphonuclear neutrophilic leucocytes are inhibited from ingesting the carbon and thus the endothelium of the vascular capillaries and the monocytes of the blood can be brought out. By passing the same solution through lymph glands by interstitial injections, he then found that both the endothelium of the sinuses and the monocytes coming from them could be distinguished from the reticulum, while in the follicle proper, only the vascular capillary endothelium showed the carbon particles until stained clasmatoocytes had wandered in. He thus separated the lymphatic endothelium of the sinuses from reticulum, and the clasmatoocytes of the follicles from

lymphocytes. Kiyono (29), on the other hand, finds that with injections of other particles, for example, carmine, both the reticulum and the endothelium of the sinuses take up the particles but that the endothelium can be distinguished by the fact that it always takes up a larger amount. This is more in line with the general experience in these recent experiments in phagocytosis, namely, that the property of phagocytosis is a very general characteristic of cells, but that some strains are more highly differentiated along this line of taking up particles than others. Clasmatocytes take up and store particles to a very marked degree. In the case of the endothelium making the wall of a vessel, it must always be considered whether the granules are passing through the cells or are being stored by them. At any rate, the process of selective phagocytosis does offer a method of showing that the embryonic separation of the two types of cells in the sinuses is retained in the adult, the reticular cells making a framework, the endothelium giving rise to monocytes. Kiyono however believes that both the reticular cells and the endothelium give rise to monocytes (histiocytes).

Foot (21), using India ink as a specific granule to bring out endothelium and the cells that are derived from it, finds clumps of cells on the surface of the capillaries of the lung under the irritation of tuberculosis, which stand out by their ingestion of carbon particles. From this he concludes that clasmatocytes differentiate from endothelium in the adult. If it can be conclusively proved for the adult as it has been proved for the embryo, that endothelium keeps on giving off clasmatocytes in certain areas or can be made to do so by certain stimuli, it will be of great significance in pathology. McJunkin's experiment has the further significance of showing that the endothelium of the lymphatic sinuses reacts like vascular endothelium which is in line with the theory that the lymphatic system is a part of the venous system, its lining cells having the same fundamental origin from the angioblast stem as a differentiated cell, rather than coming from the flattening out of mesenchyme cells to line spaces.

Thus the studies of Mallory and his school on the "endothelial leucocyte" which could now better be called the "endothelial monocyte" all center around the very important question as to whether new clasmatocytes differentiate from endothelium in the adult under normal conditions or can be made to by abnormal stimuli. It seems to me likely that this will ultimately be proved conclusively.

That endothelium in the adult gives rise to the monocytes of the blood stream in certain places seems adequately proved. Thus mono-

cytes can be found so readily and in such numbers in the veins of the splenic pulp that monocytes have been called splenocytes (56, plate I). McJunkin and Kiyono have shown that the sinuses, both lymphatic and vascular, also contribute, and Aschoff thinks that the large number of monocytes found in the veins of the extremities shows that the same cells get free in bone marrow.

It will now be interesting to sum up the history and to show the very wide range of studies which have been necessary to bring out the great group of the histiocytes and to show that the monocytes of the blood and the elasmatoocytes are identical cells, histiocytes. The cell-type was first brought out by Maximow by its specific response to injury and then by its reaction to vital neutral red; then the studies of Bouffard, Goldmann, Evans and Schulemann and many others have brought out the fact of the power of these cells to phagocytize, segregate and store particulate matter. Evans then made the final differentiation of the elasmatoocyte from the fibroblast, while Cunningham has conclusively separated it from the serosal cell. The next step came in the doctrine of the origin of blood cells from endothelium by Mallory, by Schridde (64) and Schmidt (63). Then Aschoff and Kiyono showed that the monocyte in the blood stream has an identical reaction toward particulate matter (65), that the reaction to neutral red was the same. Aschoff, Kiyono, McJunkin and others have shown that the endothelium of the adult both lymphatic and vascular can in certain specific places give off monocytes, and now it has been conclusively proved embryologically that the type of the white cell that comes from endothelium is the monocyte, which is identical with the elasmatoocyte. Both cells have been seen by the writer in a living embryonic form to differentiate from endothelium and to become free, the monocytes dropping off within the vessels, the elasmatoocytes outside; but the cells are interchangeable afterwards, for the elasmatoocytes have been seen to enter the vessel and the monocytes may pass out. The entire group has a very characteristic type of motion due to a differentiation of the periphery of the cell which keeps the surrounding fluid in motion while the cell itself has but a very slow grade of locomotion.

*Origin of granulocyte.* The origin of the granulocytes can also be made out on the third day of incubation. As shown by Danchakoff (6) the granulocyte, the forerunner of the leucocytes, is always an extra-vascular cell. It appears from the mesoderm and lies near a vessel and at first cannot be told from a single angioblast. It has the same dense azurophilic cytoplasm. As soon as it has once divided however it can

be differentiated because the two granulocytes separate, while two angioblasts remain together. In the living specimen or in the fixed blastoderm this can be made out as it cannot be made out in sections because in the total blastoderm every cell of an area can be seen which gives this type of material an enormous advantage over sections. The nucleus then becomes eccentric and the centrosome is made very obvious by the development of a crescent of very fine uniform granules, also stainable in very dilute neutral red. These cells then move toward the vessel, often line up in a row in order to enter. They enter the vessels half way between the nuclei of the endothelial cells which means that they push their way between the cells. They make the wall bulge in and once I have seen the edges of the endothelial cells separate. They move very slowly, but it is a real locomotion, not the movement in one place of the clasmatocyte. There is no active amoeboid motion of these cells at this stage, meaning a typical amoeboid locomotion associated with a fluid state of the cytoplasm as shown by active flowing and streaming of granules within the cell. During the third day the granules of these cells are always still and always in a definite crescent around the centrosome. Thus the granules of the endothelial cell that stain with neutral red are larger and are scattered throughout the cell, the granules of the erythroblasts are arranged around the nucleus while the granules of the young granulocytes are arranged around the centrosome. It may be possible subsequently to show that the substance which stains with neutral red in endothelium is not present in primitive granulocytes. That is, it is possible that a further study with vital dyes may differentiate the original angioblasts from the granulocytes. The early granulocytes are all pseudo-eosinophiles, that is, they have fine granules. The eosinophiles with the granules in the shape of rods, so characteristic of the chick, develop a little later; I have not found any on the third day and but few during the first seven days. There are no cells to be made out with the basophilic granulation or mast cells and Maximow has found that they develop late in mammals.

From these observations it seems it seems fair to say that no cell has as yet been identified as a common stem cell for the angioblast and the granuloblast. Indeed it has been the contribution of Naegeli throughout to show that there is no adequate proof that the primitive form of each type of blood cell is to be identified as a common stem for all. The only proof which would be adequate of a common hemoblastic stem would be an experiment in which the stem cells in an area where they all become angioblasts normally, should be made to become

granulocytes, as for example if the single basophilic cells of the area pellucida of the chick of the second day which normally make syncytia of angioblasts could be forced to make granulocytes. This is not beyond the range of possibility as, for example, Murphy (48) found that the injection of splenic tissue into a chick stimulated a very marked formation of blood cells in the allantois and in the diffuse connective tissues of the embryo far beyond the normal limits. Thus a connective tissue of the embryo which does not normally do so can be made to differentiate into blood cells. This experiment he suggested to Madame Danchakoff and she has been utilizing it in recent studies (7).

Weidenreich (67) has given an extensive study of the granulocytes paying especial attention to the forms of the nuclei; he has shown that the three types of granular cells have characteristic nuclei. The neutrophilic leucocytes have nuclei with small lobes, often five in number, while the nuclei of the eosinophilic leucocytes rarely have more than two lobes. The mast leucocyte he has proved to be a dying cell without a centrosome, and with irregularly staining granules. In all of the chick blastoderms there are a few dying blood cells with fragmented nuclei but they are always easy to distinguish.

*Lymphocytes.* In the study of the living blastoderms up to the seventh day, which is as far as I have yet carried the work, I have not seen any evidence of a formation of the lymphocyte in the yolk sac. Further studies may bring this cell out, or it may be that the lymphocytes form only within the embryo itself. The lymphocyte appears in the circulating blood on the fifth day, in its smallest form; an occasional one may occur on the fourth day. In the living state it has a nucleus with a sharper nuclear membrane than any other cell. This is a difficult criterion to use since all nuclei become distinct as the cells die. It has a small amount of cytoplasm and a few azur granules. Lymphocytes are readily killed by too strong a dose of the dye; the granules do not stain readily in neutral red but may be made to by increasing the amount of the dye. In the stained blood smears, the lymphocytes are easily distinguishable by their characteristic nuclei and the fact that they are all at first small lymphocytes. Their origin in the embryo needs much further study, but from their late appearance as a specific cell type it seems to me that they should not be identified as a stem cell. In the general use of the term "lymphocyte" or "lymphoidocyte" as a stem cell, it has been, however, more commonly the large mononuclear form or monocyte so identified rather than the small lymphocyte.

For the adult, it is well recognized that this cell occurs in follicles in lymph glands and spleen, where it probably comes from the reticular cell, and it is swept into the blood stream in the lymphatic sinuses and the splenic pulp. Thus our knowledge of this cell in the adult stresses that it is a cell of extra-vascular origin. The stem cell of the lymphocytes is far from adequately known. It needs study both in the embryo and in smears of living cells from adult lymph glands.

The lymphocyte has been distinguished as a specific functional group by Murphy and his school (46), (47), (48), (49), (50). The lymphocyte group is more sensitive to x-rays and to radium than other normal cells. With a certain dose it is the first normal cell injured, with a less dose it is stimulated. Murphy has demonstrated a specific relation of lymphocytes to immunity toward tumors in the chick, by showing that before there is any great mass of lymphocytes, that is, on the eighteenth day when they form in large numbers in the spleen, chicks are susceptible to tumors to which hens are immune. Then he has killed the lymphocytes of the hen by x-rays and produced susceptibility to the tumor. These studies are still being carried on by Murphy and are one of the very valuable contributions of modern medicine.

The entire absence of autolytic and peptic ferments in the lymphocytes separates them from both leucocytes and from monocytes.

#### CONCLUSIONS

In stressing the value of embryology as a part of the newer form of the experimental work on blood we may call attention to this advantage, that each cell as it first differentiates is specific, for example, the erythroblast of the second to the fourth day with its rosette of granulations arranged with reference to the nuclei. The erythroblasts have however a long period of maturation before they become the red blood corpuscles of the adult stage. The plasmatocytes and identical monocytes on the third day have a characteristic segregation apparatus, and peculiar type of motion; the granulocyte on the third day has its specific type of granulation, chemotactic response and characteristic arrangement of the granulation to the centrosome, and finally the small lymphocyte on the fifth day has its peculiar nucleus. That subsequently, when all these types of cells are dividing, it becomes difficult to distinguish all the young cells, the whole history of hematology attests and I doubt if every type of cell in adult bone marrow can be identified

before completion of the study of developing blood in the entire cycle of the embryo with vital methods has been accomplished, especially studying the cells just after division.

It is now possible to suggest as a working basis for further study, that there are three strains of cells of the connective tissues which contribute to the blood cells. All of these strains give more cells to the connective tissues than to the blood. They are the angioblasts which give the endothelium, the erythrocytes and the monocytes; of these the red corpuscles are all intra-vascular unless the extra-vascular origin of red cells in bone marrow of mammals proves correct. The red cells have a specific granulation which can be stained vitally and is arranged around the nucleus. The monocyte stem is largely extra-vascular, in small part intra-vascular, giving the histiocytes which are the monocytes of the blood and the clasmatoocytes of the connective tissues. They have developed along the line of the phagocytic power of their parent cell, endothelium, and are especially differentiated to take up and store particulate matter. It has, I think, become clear that certain endothelial cells, like the Kupffer cell of the liver, which is a specialized endothelial cell within the capillaries of the liver anchored out into the blood stream by guy ropes of cytoplasm, and the endothelial cells of the veins of the splenic pulp as well as the endothelium of the splenic sinuses have the maximum power of phagocytosis and that they clear the blood stream of foreign particles within a very few minutes. Such foreign particles are constantly excreted by the kidneys but if more particles are introduced than the kidney cells can excrete for the time being, the clasmatoocytes of the connective tissues take up the dye, store it and eventually give it back for excretion. It is obvious that the amount of dye in the experimental procedures is very far in excess of any usual amount of foreign material to be found in an organism; moreover the substances used in the experiments are all insoluble. These same cells take up red blood cells and cell débris. How extensive their function and how related to normal metabolism in the taking up of substances which they can dissolve is not known. Shipley (64a) regards their vacuoles as organs of digestion.

The second strain is the granulocyte. These cells, certainly the neutrophilic leucocytes, are also phagocytic but they are developed especially along the line of a true amoeboid type of motion with fluidity of the cytoplasm and marked flowing of the granulations associated with great speed of locomotion. They develop specific types of granules which are at first very definitely arranged in a crescent around the

centrosome. They are specifically sensitive to certain chemicals, that is to say, they have a high degree of chemotaxis. The small amount of knowledge we have of these cells seems to verify Ehrlich's theory that their functions are related to their specific granules, since it has been shown that the neutrophilic leucocyte has a proteolytic action probably brought out when the granules are set free from the cell. Thus pus from neutrophilic leucocytes becomes fluid. The neutrophilic leucocytic cell responds to certain known stimuli such as the pyogenic bacteria or their toxins, the eosinophilic to certain others such as those produced by parasites. The mast cells are the least understood and are granulocytes which develop late and remain extra-vascular, since the mast cells of the blood have been shown by Weidenreich to be degenerating cells and not true mast cells. Mast cells occur in the bone marrow, along blood vessels and between muscle fibers; their function is totally unknown.

The third strain is the lymphocyte which is the last to develop and is exceedingly widely distributed. It occurs in lymph glands, hemal glands and spleen, that is, in a reticulum near lymphatic or vascular sinuses by means of which it enters the blood stream. It also occurs in small follicles near lymphatic capillaries and ducts or even in small clumps without such relation very widely distributed throughout the organs and in bone marrow as well. Thus it may occur wherever there is reticulum. In fact it makes the third great stem of the diffuse connective tissue cells, which are clasmatoocytes, fibroblasts and small round cells or lymphocytes.

Thus the separation of these three strains of white blood cells by the embryological method seems to correspond with functional groups as far as they are yet vaguely known, the leucocytes being highly amoeboid types, with specialized chemotactic responses and functions probably related to their granulations, the monocyte stem with its specialized powers of phagocytosis and the lymphocytes with their marked sensitivity to x-rays and their relation to certain forms of immunity.

The sharp separation of the blood-forming organs into two groups, bone marrow and lymph glands, as Ehrlich thought, has not been borne out. In the first place, blood is not so sharply separated from the connective tissues in general. In the embryo the origin of blood from the connective tissues is very widespread and can be made much more so by certain experimental stimuli. In the adult the location of the differentiation of blood cells is much more restricted. Two cells are necessary, reticulum and endothelium. The reticular cell in its rela-

tion to blood needs much more study. Under normal conditions the origin of the red cells is restricted to the bone marrow, the origin of the histiocytes of the blood takes place certainly from endothelium in certain special areas in the adult. How much the differentiation of new plasmatocytes goes on normally in the adult or how much it can be stimulated, is not yet clear. The origin of granulocytes cannot be regarded as restricted to the bone marrow when one considers the eosinophiles and the still more widely distributed mast cells. Lymphocytes arise in the bone marrow as well as in all so called lymphoid tissue.

The study of the blood at the present time has passed beyond the morphological stage and is a part of the development of modern experimental cytology. In this study the anatomist, the physiologist and the pathologist meet on common ground.

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## ARTERIOSCLEROSIS

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The following review of the present status of our knowledge of arteriosclerosis was attempted unwillingly for it must be apparent to all that most of the extensive literature upon this subject consists of reviews. These are based upon the few papers which represent real study of the anatomical and functional changes, so that apart from the conflicting results of these few investigators one is impressed by the feeling that most of what is written has only the value of opinion and that we are extraordinarily ignorant with respect to this common affection. Diametrically opposite views are put forward as to the causes, the anatomical nature and effects of arteriosclerosis, many names of different significance are proposed for it and withal there has been no unanimity of opinion as to what should be included under this heading and what excluded.

For the purposes of this review I shall use the term arteriosclerosis rather than atheroma, endarteritis deformans, atherosclerosis or any of the other terms already proposed, simply because it is the most familiar and best established and because it expresses accurately enough our very inaccurate knowledge of the condition.

For the purposes of this review we must exclude from consideration syphilitic aortitis and arteritis, distinctly infectious processes in the arteries caused by other organisms such as the tubercle bacillus and the unknown cause of periarteritis nodosa, traumatic and toxic injuries leading to thrombosis and healing processes in the vessels, and perhaps with less justification, all the forms of obliterating endarteritis which cause the occlusion of the vessels of the extremities and of organs.

Arteriosclerosis remaining for consideration here is an affection of the arteries whose cause is not yet clearly known. It is characterized by constant anatomical changes of which the most conspicuous is a thickening of the intima accompanied by fat deposits. Changes in the muscular tissue of the media and in the elastic tissue are sometimes extreme but often very inconspicuous. These changes indicate a deterioration in the functional capacity of the walls and are followed

by necrosis, calcification, etc., which are secondary changes but which generally constitute the most obvious alteration in the diseased vessel wall.

Marchand sums up arteriosclerosis in its wider sense as all those changes of the arteries which lead to a thickening of the wall, especially of the intima, in whose development degenerative changes (fatty degeneration with its results) sclerosis and calcification (including calcification of the media) arise together with inflammatory and productive processes.

It must be remembered, however, that while all students of arteriosclerosis recognize the occurrence of lesions in the various coats and tissues of the vessel they are far from unanimous in their conceptions of the order in which these arise and of the relations which they bear to one another. For some the lesions of the media are primary, those of the intima secondary or compensatory—for others the reverse is true. Equally discordant opinions are expressed as to the disturbances of function which result from or are the causes of these changes. The time is ripe for a searching new study of the whole matter, but little can be expected from a review.

Nevertheless, the available material may be considered under three heads:

1. The anatomical changes in the vascular system.
2. The associated functional disturbances.
3. Etiology.

1. *The anatomical changes in the arteries.* The current descriptions of the histological structure of the arteries leave much to be desired (Renault, Kölliker, Ebner, Grünstein, Klotz) but in so far as this concerns the arrangement of the musculature and elastic tissue it is due, as v. Ebner points out, chiefly to the great variability of structure in different parts of the arterial tree. Not only are there constant differences among the arteries but the structure of the wall at the same point in a given artery may vary in different individuals. It is with regard to the minuter structure of the intima, however, that most uncertainty persists.

In general the aorta and those trunks which developed in the branchial arches maintain a lamellar arrangement of the elastic tissue in the media while such arteries as the coeliac axis, the superior and inferior mesenterics, renal arteries, common iliaes and femorals present a media composed essentially of smooth muscle with a cobweb-like arrangement of delicate elastic fibrils which run in all directions, while

the denser elastic tissue is relegated to the adventitia. The popliteal and tibial arteries are said to be exceptions to this rule but this is not true, and while the brachial arteries for some distance show lamellated elastic tissue in the media, the more peripheral parts of the arteries of the arm have the structure of those of the leg. This lamellated structure of the media is also found for a short way in the proximal parts of the intercostal arteries and exquisitely developed in the pulmonary artery.

Longitudinal tangential section of such a vessel with lamellated elastica in the media shows very plainly the flat spiral arrangement of the smooth muscle fibers which alternate their direction in successive layers so that in the section they appear to take a herring-bone arrangement. The laminae are supplemented by numerous fibrils of elastic tissue which accompany the muscle fibers. Tangential sections through the media of vessels which have no lamellated elastica show no such rapid alternation in the direction of the muscle fibers. In them (renal) there are sometimes bundles of longitudinal muscle fibers outside the media accompanied by the longitudinal elastic fibers of the adventitia.

The elastic tissue of the adventitia is chiefly represented by longitudinally placed fibrils which take a steep spiral direction but in such vessels as the superior mesenteric and coeliac axis there is generally a quantity of elastic tissue coursing in a circular direction as though pushed out from the media. An external elastic lamella is described in many vessels but it is usually not a distinct lamella but only the last lamella of the media or the innermost elastic layer of the adventitia. The development of adventitia varies greatly in different tracts of the vascular tree. It is important in carrying the vasa vasorum but little attention has been devoted to it otherwise in the consideration of arteriosclerosis. Longitudinal muscle bundles are sometimes a very prominent constituent.

In general the intimal coat has been the center of interest. There is no difference of opinion as to the constant endothelial lining except that some early authors attempted to show the origin of the connective tissue layers from these cells, an idea easily dismissed by Marchand. The tissues outside the endothelial layer and within the media have been discussed by Langhans, Kolliker, Ebner, Eberth, Ranvier, Key Aberg, Jores and many others, and a fair unanimity of opinion arrived at, although much of the earlier work was done in the lack of all the more recent staining methods. Key Aberg described the internal elastic lamina with its associated longitudinal smooth muscle fibers

and the innermost layer of a tissue more difficult to interpret. This is composed of a finely fibrillar or granular ground substance in which lie the cells first clearly described by Langhans although pictured also by Virchow. These are much branched cells, sometimes very large and stretching far and wide their long processes which, like the rest of the cell body, often contain granules which are probably fatty. He distended with an asphalt-chloroform injection mass a network of canaliculi which correspond fairly well with the outlines of these cells. Similar canaliculi could be injected in the media but in both cases his drawings remind one of the artificial spaces which can be formed in a similar way in the cornea and scarcely suggest preformed spaces. In several attempts I have so far not succeeded in injecting india ink into any spaces in the thickened intima of a sclerotic aorta but once air was driven into the tissue so as to distend a network of tiny spaces.

Jores devoted a great deal of space to the consideration of the histology of the intima of the aorta and recognized a musculo-elastic layer (Thoma) composed of a thin outer and a thicker inner layer of elastic tissue between which were the longitudinal muscle fibers. Inside this he describes a fibro-elastic layer in which laminae or fibers of elastic tissue derived from the internal elastica are interspersed with connective tissue elements. Still nearer the lumen is a layer of almost pure connective tissue.

In my own observations the variation in the structure of the intima is so great in different vessels that any single description seems conventional. It is true that at the inner margin of the media one can generally distinguish a musculo-elastic layer which is composed in its most typical form of an outer single smooth lamina of elastic tissue, a layer of longitudinal muscle fibers and an inner layer of elastic tissue, less simple than the outer and clearly made up of a network of fibers or laminae. There must be a chemical difference between these two elastic structures for the outer stains red when the inner fibers are blue, or brownish-red when they are purple. The inner layer is often in immediate contact with the endothelium in smaller vessels. It is not a single lamella as a rule, although it may appear so in places, but tends to split up into a whole series of lamellae or rows of fibers. Jores' emphasis of this as a hyperplastic or hypertrophic process seems labored when it is so generally present and it is so difficult to find any part of any artery in which it forms a single lamella. Other finer elastic fibrils spread away into the innermost layers of the intima. There seems to be no pure connective tissue layer on this account and in

any case the differential stains do not bring out much connective tissue in characteristic form in the intima. In a section stained by Van Gieson's method the intima has a vague purplish color in great contrast with the sharp red of the adventitia. The cells are extremely inconspicuous until arteriosclerotic changes arise. Under those conditions they can be described in detail later. Such cells as are seen in the normal intima have the appearance of smooth muscle and are often seen to be continuous with the smooth muscle of the musculoelastic layer. However, this is a point left unsettled by Langhans, Key Aberg and all the others. The tendency is to regard these branching cells as of connective tissue origin although it is impossible to decide on morphological grounds alone. It is, however, evident that the elements of the internal elastic lamella offer no obstacle to the extension or growth of tissue from the underlying media into the region of the intima and it seems unnecessary to ascribe the origin of all tissue found in the thickened intima in arteriosclerosis to the cells of the intima itself. Indeed, cells appear there which could hardly have originated in the intima.

On such a basis of normal anatomy the descriptions of arteriosclerosis have been constructed. It is possible to pass over the early descriptions of Rokitsansky and Virchow and even those of Koester, who appears to have described the mesarteritis of syphilis. Nevertheless, we shall have to recur to Koester's ideas of the part played by the vasa vasorum. Marchand, Ribbert, Reich, Thoma and others have opposed him on the ground that arteriosclerosis is not an inflammatory process in the precise sense of the word but rather, as Marchand said, a nutritive disturbance of the vessel wall followed by sclerosis and degeneration.

Thoma has made himself conspicuous by his long campaign in favor of a mechanical origin of the condition. His theories are hardly generally accepted now but there are some adherents. In principle he thinks the whole process dependent upon a localized or more widespread weakening of the muscular media which bulges outward, thus bringing about a slowing of the blood stream in the widened area. This, by some means not clearly explained, leads to a new formation of tissue on the part of the intima, sufficient to fill up level the depression produced by the bulging of the media. When the artery is laid open this patch of fibrous tissue is thrown into relief by the turning outward of the elastic wall—but if the artery be injected with paraffin at the blood pressure, the cast on cooling will show a smooth wall.

Every point in Thoma's argument has been pretty surely demolished by his critics. The weakening of the media is not obvious until a

late stage is reached and then it may well be the effect of the dense plaque of tissue lying upon it. There is no cogent reason to believe that a slight dilatation would be compensated by the new formation of tissue. If it were, it ought to be formed as in the case of the granulation tissue which replaces the stagnant column in a ligated vessel. In that case the mode of development of elastic tissue should be Jores' regenerative type and there is difficulty in understanding the colossal amounts of fat which are lodged in the tissue from the beginning. Thoma begins with the scholastic argument that in the aorta of the embryo there is no connective tissue intima and that it is the closure of the ductus Botalli and the umbilical arteries that brings about a change in pressure in this portion of the circulation which thus becomes too wide and must be adapted by new formation of connective tissue in the intima. This is the same principle which he uses in explaining arteriosclerosis when he claims that a slowing of the stream from local dilatation produces a compensatory thickening and releveling of the wall.

Jores writes at very great length on what is represented as a totally new attitude toward arteriosclerosis which consists in distinguishing at first in smaller and then in larger vessels between two modes of formation of new elastic tissue in the intima. Jores' earlier studies had shown that elastic tissue in the form of very fine fibrils could be formed afresh in new connective tissue. This process he recognizes now in the tissue which arises in the organization of a thrombus in a vessel or in that which brings about the obliteration of a small artery. He names it the regenerative form of connective tissue growth of the intima and contrasts it with another which he calls hyperplastic thickening of the intima. The latter consists in the appearance of many lamellae or filaments of elastic tissue within the internal elastic lamella which he thinks are split off from the original lamella. He admits that much of this is obvious under physiological conditions but that it becomes emphasized and is the predominant character of the pathological thickening of the intima.

Of course, the occurrence of these two forms or states of division of elastic tissue is true but it seems to throw no light on arteriosclerosis to distinguish them, for one is a characteristic of the formation of new actively growing tissue, the other is formed normally and is only made more conspicuous by the separation of the fibrils or lamellae by the increase of other tissue in the arteriosclerotic plaque.

It seems difficult for Jores to bring himself to make the statement that this is an abnormal condition and he contents himself with say-

ing that it is a departure from the physiological state. All the rest of his suggestions about the effects of high blood pressure in augmenting this and the effects of strains which bear upon the longitudinal muscle seems pure speculation. He does, however, recognize the fact that degenerative changes with the accumulation of fat assume a primary place in the development of arteriosclerosis.

Voigts, in his dissertation, set the matter of the distribution of elastic tissue and connective tissue in the intima in a clearer light and showed, as described above, that the elastic fibers inside the musculo-elastic layer are abundant and form no definite lamellae but only networks. Jores had shown that fine fat droplets adhere to the elastic tissue of the intima except the actual internal elastic lamella and also involve the media. This is readily seen in any frozen section from a sclerotic vessel stained with Sudan. A fine and diffuse red brown staining is found in patches in the media and in the intima, but while the wavy lamella which forms the outer limit of the musculo-elastic layer is unaffected, the palisade-like row of fibers which forms its inner limit is finely strewn with red globules. Torhorst thinks this is not a deposition of fat in the elastica itself but rather in the interstitial supporting substance.

Disintegration of the elastica has been described by Manchot and others. Dmitrijeff describes degeneration and disappearance of fibers and new formation of elastic tissue in the course of arteriosclerosis. Matusiewicz also describes and illustrates the more isolated calcification of portions of the elastica in the arterial wall and McMeans discusses in great detail the degenerative processes in the elastica associated with splitting and breaking of the fibers.

Such theories as that of Thoma which ascribe the sclerotic changes to localized dilatation through weakening of the media, must refer this weakening to the smooth muscle or to the elastic tissue of that wall but definite observations of alterations in either of these tissues except in late stages of the disease are hardly to be recognized. It is of course true that under thick degenerated sclerotic patches one ordinarily finds the media stretched thin with perfectly straight and rigid looking elastic lamellae and very much attenuated muscle fibers all pressed together into a mass whose details are indistinct. In earlier stages it is possible to find many breaks in the elastic lamellae but it is difficult to avoid the idea that these may be due to artefact in making the section—nevertheless, in many cases on tracing the homogeneous wavy elastic lamella that bounds the musculo-elastic layer toward

the media (the true internal elastic lamella) breaks are found which cannot be due to artefact for from the separated blunt ends of the broken lamella there spring delicate elastic fibrils which bridge the gap. Destructive changes in the muscle of the media are rather more definite when they are found in a section in which the elastic tissue is stained for they then appear as patches filled up by many collapsed elastic lamellae between which no traces of muscle remain. The granular degeneration of elastic fibers described by Jores, Weizman and Neuman, is regarded by Voigts as not an abnormal condition.

Ribbert expresses the idea that inasmuch as some areas of the best arterial wall may be weak and easily bulged out by a high blood pressure, it might readily follow that the corresponding area of intima becomes stretched over this so that its meshes are laid open and soon filled with plasma pressed in from the blood stream. This might account for the hyaline edematous appearance of the plaques thus formed and perhaps also for the advent of fat which is so uniformly present. Nothing in this idea seems plausible for it is difficult to imagine such a power of resistance in the intima, and further difficult to understand why it does not protect the underlying media from the distending force. The imbibition of blood fluid which may well occur could hardly give rise to all the extraordinary new tissue which forms the plaque and which, if we may believe Reich's statements, is not necessarily formed in the dell of an area of stretched out media since he finds the internal elastic lamella fixed in corrugated form between the plaque and the media while the normal elastic lamella stretches out smooth when the artery is distended.

Alb. Aschoff and also L. Aschoff trace in detail the gradual development of the intima with its increasing elastic tissue from early youth to mature age. After this there is a period of quiescence which is then followed by another period of advancing senility in which no more elastic tissue is formed but much new connective tissue. This leads the latter to the view that the loss of elasticity is the basis upon which a compensatory strengthening by the formation of new connective tissue occurs, an idea which is not very different from Thoma's. Klotz, too, in a long and careful paper upon the importance of medial alterations, concludes that sclerosis of the vessels is largely dependent upon disease of the media brought about by infections, poisons, work or old age.

The discussions of the actual anatomical changes in arteriosclerosis have brought forth little that is new. Interest centers especially in

the intima although Thoma and his students, Klotz and others, conscientiously search for primary alterations in the media. Aside from the extensive changes in the media of the peripheral vessels which are quickly followed by calcification or bone formation and scarring with changes in the intima which may be comparatively insignificant or which may have the character of an obliterating endarteritis, the media in the aorta and visceral arteries seems relatively little changed in comparison with the extent of the internal alterations. Such changes as there are may be recognized by measurement of the thickness of the media or by the finding of actual degeneration and disintegration in the muscle and elastica beneath the plaque, always suggesting that they may be secondary to the presence of the plaque, as easily as its cause. Little attention has been devoted to the adventitia but Klotz and Adami point out that this coat too is sometimes thickened opposite an arteriosclerotic plaque. I have seen this also but it is so irregular and the tissue so loose that it is hard to believe that it really affords protection to that area.

In the intima Klotz has studied especially the fatty streaks which are so familiar and which he and many others describe as occurring in the same distribution as the earliest arteriosclerotic lesions in the aorta. This I think is not true for the yellow flecks as a rule form an almost continuous line along the dorsal intima of the aorta and circling round the innominate or carotid while the actual sclerotic plaques are quite irregularly placed, often, however, surrounding the orifices of the intercostal arteries. These fatty flecks have been thought by many to be the transient effects of infection or intoxication, disappearing after convalescence. This is of course difficult to prove but it seems plausible. Klotz has described them minutely showing that the fat, some of it anisotropic, is accumulated in cells resembling lutein cells which Anitschkoff calls cholesterolin phagocytes, and also in other larger branching connective tissue cells. The greater part is apparently taken up by the cells of the first order which are round cells with abundant protoplasm loaded with oil globules, and a small deeply stained nucleus. They are clustered loosely in clefts in the superficial intimal tissue. The branched cells which contain fat are marked out to their distant branches by the fine globules.

There is no sharp line to divide this earliest condition from later stages in which there is associated with these fat accumulations a considerable new formation of tissue including elastic tissue fibrils. The later anatomical changes seem to consist in further accumulations of

fat, in the multiplication of connective tissue cells and to a slight degree of fine elastic fibrils, in the appearance of much new hyaline fibrous tissue with a great deal of material which microscopically has the appearance of coagulated fluid. Necrosis and disintegration of the tissue about the fat occurs and as the fat has by this time spread into the musculo-elastic layer and even into the media these tissues tend to be involved in the disintegration. But below the accumulation of fat and more especially above it, the newly formed tissue becomes dense and pearly so that the fat may not be visible through the plaque thus formed. The later alterations which result from the decomposition of the fats, glycerin and cholesterin esters and lecithids, are well known. Cholesterin crystals appear in the formless mass and calcium is deposited in the form of carbonates and phosphates. Klotz has explained this as the result of the formation of calcium soaps and their later decomposition with the production of calcium phosphates and carbonates but Baldauf and Wells object to this theory on the ground that they can isolate no calcium soaps from sclerotic arteries. It seems a plausible idea, however, and the soap stage must at best be very evanescent.

The dense pearly tissue which forms the plaque over (and under) the necrotic mass (atheromatous material) in which the fat lies is interesting on account of its microscopic characters. It does not give very clearly the reactions for connective tissue with Van Gieson's stain nor with Mallory's aniline blue. The bands of material of which it is composed are hyaline and do not look like connective tissue. There are peculiar long branching tubules with dense refractive walls which with the intervening and differently staining hyaline material make up the mass. In each of these is an elongated cell probably the branching cells described by Langhans and Key Aberg. It seems most reasonable to regard these as connective tissue cells but their nature is by no means certain.

Although Klotz and many others draw a distinction between the arteriosclerosis of the small vessels and that of the aorta, it seems that exactly the same changes are to be found throughout. If they refer to the Mönekeberg type of medial sclerosis, the distinction is plain, but it seems that the arteriosclerotic changes which occur in the aorta are also in most cases to be found in the peripheral vessels.

*The associated functional disturbances.* Under this heading we should consider the distribution of the diseased condition of the arterial walls, the effects upon the circulation and the heart, and the effects upon the tissues supplied by the altered arteries.

Most authors (Stengel, Brooks, Ortner, Perutz, Goldscheider and many others) lay stress on the localized occurrence of arteriosclerosis in certain areas leading to the production of symptoms which may be ascribed to the condition of the circulation in those vessels. Doubtless if this is true at all it may be true of many organs but emphasis is laid upon the existence of localized sclerosis of the arteries of the brain, of the heart, the kidneys and of the intestinal tract. In the case of the brain, aside from actual hemorrhage from ruptured arteries, transient losses of function are often ascribed to a spasm of the arteries or to temporary inadequacy of the blood supply on account of the narrowing of the arteries. There seems to be no convincing proof of the occurrence of a spasm which might produce such a cessation of function and even when there are obvious encroachments upon the lumen in the arteries the explanation of a temporary lapse is difficult. The whole subject of angina pectoris which is associated with sclerosis of the coronary arteries is too extensive to consider here. There as in the case of the crises of pain which sometimes accompany arteriosclerosis of the mesenteric vessels, opinion varies as to whether pain is due to a cramp of the vessel walls or to the sudden ischemia produced by such narrowing.

With regard to localized arteriosclerosis of the branches of the renal arteries, there is a great literature and recently (Lohlein) even a further localization in the arterioles of the glomeruli is held responsible for many of the disturbances in function. Jores finds that sclerotic changes with hyperplasia of the internal elastica are not present in all cases of contracted kidney in chronic nephritis nor in the acute and subacute forms in which there is already high blood pressure. But this too is a subject which cannot here be considered in detail.

With regard to the effect of general and localized arteriosclerosis upon the circulation there has been much speculation without as much actual observation and experiment.

The arteries become rigid, lose their distensibility and resilience, lose to some extent their muscular contractility and are in addition in some cases dilated and deformed, in others intermittently or extensively encroached upon by thickenings of their walls. One may frequently observe this last fact in the coronary or mesenteric arteries in which, while much of the artery is thin walled and dilated, there are marked constrictions at certain points produced by thick projecting plaques of sclerosis. These are, in general, affection of the larger and medium-sized vessels. The very small arterioles and of course

the capillaries are in general less affected although in some localities, as in the kidneys, they are definitely thickened and narrowed.

The effect of these changes upon the general arterial blood pressure is one point which has been discussed; the effect upon the rate of delivery of blood to the tissue, the other.

Romberg states that the very rigidity of the walls interferes with the passage of the blood at the normal rate, while Allbutt opposes the idea that this can offer any great resistance and thus bring about a hypertension. Hasenfeld put forward the idea that arteriosclerosis of the splanchnic arteries might oppose a great resistance to the heart and it was actually shown by Longcope and McClintock that ligation of the splanchnic arteries produced a marked rise in blood pressure, while obstruction of other arteries had little or no effect. But Marchand showed that there is little relation between arteriosclerosis of the splanchnic arteries and cardiac hypertrophy. In general, it is difficult to correlate high blood pressure and cardiac hypertrophy with arteriosclerosis. In an analysis made by H. P. Smith of seventy-two cases at autopsy, chosen because the blood pressure was high, it was found impossible to show any parallelism between arteriosclerosis and blood pressure, while there is a very marked parallelism between the curves indicating the degree of nephritis, hypertension and cardiac hypertrophy. The lack of the auxiliary contractility of the arteries in driving on the blood into the capillaries, the lack of distensibility of the larger arteries, and the actual narrowing of certain points in many arteries by encroaching plaques, must offer some increased resistance to the heart but it seems insignificant in its effects upon the blood pressure and upon the heart itself when contrasted with the overwhelming effects of chronic nephritis. Heightening of the blood pressure seems to be a phenomenon actively produced by the circulatory apparatus and not the passive result of changes in the walls of the arteries.

With regard to the changes in the amount of blood delivered to the tissues by sclerotic arteries, we have no actual figures but there is much indirect evidence of a somewhat unsatisfactory kind.

The part played by sclerosis of the coronary vessels in the production of myocardial scars has been much discussed and all the evidence tends to show that (Fujinami, Strauch) in most cases at least the coronary branches leading to the scarred areas are not sclerotic. Nor is the matter made clear by the assumption that narrowing of the ostium of the coronary is responsible. In many cases the affected areas of the heart wall are in places scar-like, in other places soft, translucent and

deep grayish-red. These seem to be early stages in the formation of a scar—the heart muscle fibers are lost but the granulation tissue which replaces them is extremely vascular. This in itself is inconsistent with the idea of scar formation on account of anemia, and probably is only one representative of the large group of cases in which causes other than restriction of the circulation (infections, toxins) have destroyed the myocardium.

Sclerosis of the vessels of the kidney in association with scarring of the kidney substance presents exactly the same problems. The statement is rather dogmatically made that sclerosis of the fine branches of the renal arteries is the cause of the degeneration and scarring of the kidneys in certain cases. Side by side with these are cases in which the kidneys are equally scarred and degenerated, in which there is little or no arteriosclerosis. It is as difficult to accept the idea that the arteriosclerosis causes the shrinkage of the kidney as the reverse. Some light seems to be thrown upon this by the indisputable instances in which the loss of function of an organ is followed by changes in the arteries which lead to their narrowing by a process of obliterative endarteritis and whether the destruction and loss of tissue be brought about by the climacterium or by age or by disease, there is the correlated effect upon the supplying arteries.

*Etiology and pathogenesis of arteriosclerosis.* Arteriosclerosis is one of those diseases difficult to explain because it develops so slowly through long years of life during which a great many possible causes have had an opportunity to affect the tissues. Our brilliant discoveries as to the etiology of disease come most readily when the transition from health to a pathological state is sudden and easily recognized by symptoms. Hence every conceivable idea has been expressed and tenaciously maintained with regard to this condition and many of them are so vague and ill-supported that it is wearisome to discuss them. None, however, is clearly demonstrated to have a definite bearing on the etiology of arteriosclerosis and we are quite as ignorant of its underlying cause as were our forefathers in the days of Morgagni.

If, however, we rehearse them once more it will be seen how little actual proof there is for the efficacy of these things in producing the disease. If we set them down in a table with the names of those who have expressed themselves for and against each theory, we find the columns about equally filled with names.

More old age can hardly be regarded as a separate factor in producing this condition since it is necessarily complicated by many other

possibilities. Arteriosclerosis does not steadily increase with age as is shown by the analysis of many cases at autopsy by decades, although it may well be said that those dying in the later decades are in a sense selected persons who did not die of arteriosclerosis. It is found, however, in children and in a particularly highly developed form in young people. Romberg and Bäumler set aside old age as an essential factor.

*Hereditary tendencies* have been emphasized by Osler, Romberg, Fraenkel, Kisch and Hirsch but not regarded as important by v. Schrötter, Thoma and Edgren. Doctor Osler spoke of the quality of the arteries with which one came into the world.

*Hard muscular work* has been thought to favor the development of arteriosclerosis but the evidence is contradictory. While Weiss found it present in workers in foundries and factories, Edgren could not confirm this in farm laborers who are supposed to work hard. Of course, this too is not an isolated factor because hard bodily labor usually brings with it exposure to unhygienic housing conditions, abuse of alcohol and tobacco, syphilis and other infections and even in certain trades to toxic influences such as the fumes or dust from metallic and other poisons.

It is assumed to act through a heightening of blood pressure. Perhaps a rather pure example is that of Marchand who found in a young person who stood on one foot, the other leg being paralyzed, a marked sclerosis of the vessels of the overworked leg.

*High blood pressure.* This brings up the factor of heightened arterial pressure which is by one group regarded as the intermediary through which many of the other influences act. Sir Clifford Allbutt lays great stress upon the separation of those cases of arteriosclerosis which occur under conditions of heightened blood pressure (hypertension) from those which occur in its absence and are found without hypertrophy of the heart usually in persons of advanced age (decreascent form). He does not believe that the arteriosclerosis itself is the cause of hypertension nor would he insist that hypertension is always followed by arteriosclerosis but without giving a convincing histological basis for their separation he maintains the distinction between these forms. It is of course common enough to find arteriosclerosis in cases of hypertension even without renal disease but as Lubarsch and Pal have shown and as we have all observed, there are cases of extreme hypertension without any arteriosclerosis. Where hypertension is local, as it can be in only a few places, as in the pulmonary artery in mitral stenosis, or when a communication between aorta and pulmonary artery

is formed by the rupture of an aneurysm or by the ductus arteriosus in malformed hearts, we usually find sclerotic plaques in the vessel wall. Ljungdahl has studied this condition in many cases and finds that it is chiefly developed in those instances in which the artery is functionally overstrained. Mitral stenosis, congenital malformations and extreme pulmonary emphysema are the principal causes.

On the whole there is a widespread idea that heightened tension from whatever cause is a fundamental factor in the production of arteriosclerosis. It is assumed in the experimental studies carried out with adrenalin and with mechanical means of raising the pressure, it is used to account for the position of sclerotic plaques at the orifices of intercostal and other vessels, but in no case is it quite clear that the increased tension plays an essential part. On the contrary we find cases of nephritis with long standing high blood pressure with little or no arteriosclerosis, although hypertrophy of the muscular coat may exist. Klotz attempted to produce a work sclerosis by raising the blood pressure in the carotids and thoracic vessels by hanging a rabbit upside down each day for a long time. He succeeded in causing thereby an increased pressure in these vessels and extreme sclerotic changes. Others (Steinbiss, Miner Hill) failed to produce any changes by such means and Klotz himself did not always succeed. Harvey reached similar results to those of Klotz by compression of the aorta in a rabbit.

Apparently the element of hypertension does not enter into the conception of arteriosclerosis as a wear and tear disease of the vessels in which elastic tissue is replaced by connective tissue. Most of the names are associated with this idea from Rokitansky and Traube to Aschoff, Jores, Romberg and others. No one could deny the probability that such a replacement may occur, but the typical appearance of arteriosclerosis in the young with all its characteristic features makes it seem that this process has more individuality than mere wear and tear.

The same criticism may be offered for the expression, "increased functional demand" which is used by several to mean that under the stress of unusual activity the vessel wall suffers.

Numerous authors (Fraenkel, Huchard, Romberg, Weiss, Stengel) speak of mental activity or overactivity, mental diseases and various nervous disturbances as possibly productive of arteriosclerosis. That they may have an indirect bearing through disturbance of metabolism, or that they may be the expression of some intoxication which lies at the root of the arteriosclerosis, seems more plausible.

The association of certain diseases with arteriosclerosis is discussed but without producing very convincing evidence in its favor. Thus Huchard, Lancereaux, Fraenkel, Schrötter ascribe to gout a part in its production and others think that the same may be said of diabetes, chronic nephritis, obesity, etc., but in each case there are quite as many to deny the relation.

There remain perhaps the most important etiological factors—*infections, intoxications and unbalanced diets.*

The French school, including especially Huchard, have emphasized the importance of infectious processes in giving rise to arteriosclerosis. Typhoid fever, rheumatism, scarlet fever, diphtheria and influenza are especially frequently mentioned as being followed by the appearance of sclerotic lesions of the aorta. Thayer and his assistants, Brush and Fabyan, studied the cases of typhoid fever recalled for examination months or years after convalescence and found that they presented thickening of the peripheral arteries in a much higher percentage of cases than the controls; and at autopsy he found in deaths from typhoid fever fresh sclerotic plaques in the aorta and coronary arteries. Earlier workers, such as H. Martin, Therese, Simnitsky, had recorded observations pointing to the occurrence of lesions in the arteries after infections which might possibly lead to arteriosclerosis. Wiesel studied a large number of cases in young persons who had died of acute infections and found minute necroses in the media which sometimes showed as sunken spots in the lining of the vessel. Frothingham made similar observations in diphtheria, typhoid fever, glanders and other diseases. These papers have been reviewed by Ophüls in an excellent monograph on the relation of arteriosclerosis to infectious disease in which he shows by the careful study of five hundred cases that arteriosclerosis develops in connection with injury to the arteries resulting from various infections of which chronic septic infections of the rheumatic type play the most important rôle.

Comparison of tables made up of cases in which there was a history of one or more infections, with those made up from persons who had suffered little or no infection, shows an extraordinary preponderance of arteriosclerosis in the former.

Ophüls feels that it is misleading and confusing to consider the renal lesions so frequently associated with arteriosclerosis as a result of changes in the arteries or vice versa. They are more probably produced by the same causative agent. Nor does he regard hypertension and arteriosclerosis, which sometimes occur together, as interdependent.

Naturally attempts have been made to reproduce the arteriosclerotic lesions supposed to follow infections by experimental inoculations, either with living or dead bacteria or with their extracts or toxins, and many investigators have been able to produce lesions of the arteries rather closely resembling those of arteriosclerosis. Gilbert and Lion did this with typhoid bacilli and with an organism from a case of endocarditis. Boinet and Romary, Therese, Crocq, Manouelian and Saltykow continued these experiments. Most of these worked with rabbits and so did Klotz and Bailey, who produced distinct arterial lesions with calcification by the injection of diphtheria toxin. These animals are so prone to spontaneous arterial lesions that it seems most unfortunate that they should always be chosen by experimenters although it may be supposed that in any considerable series of positive results criticism of this sort is largely disarmed. But for this reason the injection of killed staphylococci or filtrates by Manouelian into monkeys with the production of medial lesions seems to assume a somewhat greater importance.

Nevertheless, in spite of all these experiments and these statistical studies of the co-existence or later occurrence of arteriosclerotic lesions in persons affected with infectious diseases, there is brought no satisfactory proof of the thesis that infection is the direct cause of arteriosclerosis. It seems the most plausible idea so far mentioned but it will require far more study before we see the relation clearly.

With regard to the part played by toxic substances of various sorts the most general statements are made by Huchard, Romberg, Craig, Senator and others who variously supposed the occurrence of poisonous materials capable of causing a vasomotor spasm with high blood pressure and ultimately arteriosclerosis. Craig's idea was that this was a form of auto-intoxication from the intestine. More specific arguments have been put forward by Billings, Huchard and Lunz concerning the influence of lead poisoning but Jores and Kolisko have been unable to substantiate its importance in producing arteriosclerosis. The association in this and in the following instances is so vague that it seems hardly worth while to analyze the statements more exactly. Tobacco has been ascribed the same effect by a number of well-known authors, Schrötter, Huchard, Romberg, Schott, Küllbs, and especially by Adler and Hensel. Doctor Adler was able to produce arterial lesions in dogs by the injection of relatively enormous doses of nicotine but this can scarcely be taken as a proof of the inimical effect of smoking in man. Edgren and v. Basch refuse to accept the supposed etio-

logical importance of tobacco in this disease. It is not impossible that nicotine may have some direct or favoring influence in the production of arteriosclerosis but the evidence is less than circumstantial. The same may be said of tea and coffee—there are voices for and against assigning them any importance. Alcohol has always been prominently mentioned as a causative factor usually with the further explanation that it acts by elevating the blood pressure or by causing sudden variations in the pressure. Edgren, Hirsch, Gerhardt, Schrötter, Chiari, Klemperer believed in its importance but Romberg, and especially Cabot, have been unable to show that among patients with arteriosclerosis there is any extraordinary percentage of alcoholism nor vice versa.

Since the idea that hypertension could bring about arteriosclerosis has for a long time been very familiar, it was natural that experimental attempts to test this with adrenalin, whose power of producing vasoconstriction and high blood pressure was well known, should have been attempted. Jores' experiments in this direction failed but Josue was able to cause in rabbits the formation in the aorta of localized areas of medial necrosis with dilatation and calcification. Numerous investigators followed with the same results (Erb, Pearce and Stanton, Braun, Klotz, Loeb, Githens and Fleisher, and many others). All found that areas of necrosis appear in the media which may involve its whole thickness or only a part. The artery may be dilated at this point—the necrotic tissue becomes calcified and surrounded by a granulation tissue often with giant cells, as might be the case with a foreign body. A compensatory new formation of intimal tissue which ultimately levels the intimal surface may occur. The descriptions are practically always the same and while this condition is not regarded as identical with the arteriosclerotic lesions of the human aorta, they do resemble closely the Mönckeberg type of medial calcification found in the peripheral arteries in human beings.

Such disease of the aorta occurs so often spontaneously in the rabbit's aorta that Hill thinks it necessary to have a very large series before the lesion can be accepted as the result of the adrenalin injection. But by this time the unanimous reports of many workers constitute a large series and it seems that it may be accepted that this is the effect of the injection. Whether it is the effect of the blood pressure raising influence of the adrenalin has been studied in various ways, chiefly by adding amyl nitrite or other substance which will tend to neutralize this violent pressor effect. Even then the same arterial

lesions are produced (Klotz), so that it appears that some toxic action of the adrenalin and not merely a mechanical effect of hypertension is at work.

A minute analysis of all the work upon this subject up to 1908 has been given by Saltykow and the question as to its bearing upon human arteriosclerosis seems to be settled.

Various other substances have been injected in the effort to produce experimentally arteriosclerosis, usually with the result that changes of some sort are produced but never resembling very closely arteriosclerosis. Such substances as uric acid, sodium urate, barium, ergotin, hydrastin, mercury and extracts of decomposed meat have given no consistent results.

Far more interesting are the experiments begun by Ignatowski and carried out in detail by a number of other investigators, Anitschkow and Chalatow, Starokadomsky and Ssobolew, Stuckey, Wacker and Hueck, but especially Anitschkoff, upon the effect of modifications of the diet. These modifications consist chiefly in the introduction of animal food into the diet of the vegetarian rabbit and especially in the increase in the proportion of lipoid substances. Most of these authors have ultimately investigated the effect of the feeding of cholesterin and cholesterin esters although a few have been interested in the effects of high protein diets. Anitschkow in his later paper begins by showing that the experiments of Saltykow in which staphylococci were injected over long periods were complicated by milk feeding which alone will produce the changes in the aorta accompanied by deposits of fat, while the bacteria alone without simultaneous milk feeding will not do so (Herxheimer, Reddingius and Starokadomsky).

In his own experiments in which he fed cholesterin dissolved in sunflower seed oil he thinks he has produced a condition which in respect to the deposition of fat and the hyperplasia of the intima closely resembles human arteriosclerosis in sharp contrast with the changes of the adrenalin type which bear no close resemblance to the human disease.

In these experiments, first with the feeding of cholesterin alone, then the same feeding combined with mechanical narrowing of the aorta by a ligature, then with suspension of the rabbits in inverted position (Klotz) with and without cholesterin feeding, he finds as the clear-cut result that the mechanical interference which would lead to heightened blood pressure alone fails to produce the characteristic changes in the vessel wall, while these appear when cholesterin feeding is combined with them and indeed after the feeding of smaller amounts of cholesterin.

terin than are necessary to produce atherosclerotic changes in the walls when given in normal resting rabbits. There is then something in the heightening of the blood pressure (after all no great increase in blood pressure is produced) which favors the deposition of the lipid and the development of the hyperplastic changes. Similar results are recorded for a combination of cholesterol with adrenalin injections but this seems a confusing experiment because different end results are combined.

Anitschkow's view is, therefore, that the inception of arteriosclerosis is probably to be ascribed to the storing of lipid substances in the intima but that this alone will not occur except as a process of summation with various other predisposing causes of which he has tested one or two examples. He expresses then a combination theory of the origin of arteriosclerosis in man.

#### SUMMARY

On the whole the anatomical changes in arteriosclerosis are now becoming fairly clear and there is almost complete unanimity of opinion as to what should be included under this name and what excluded. Syphilitic and other infectious forms are excluded, obliterating forms of endarteritis are usually easily classed apart with well understood cause and quite separate anatomical characters and there remains only the "ordinary" arteriosclerosis with the somewhat more obscure division formed by the medial necrosis in peripheral vessels. This form of Mönckeberg seems very distinct from the other, more nearly related to the forms of obliterating endarteritis and often combined with it. It has, therefore, been little considered here.

In the true arteriosclerosis there seems to emerge from the long continued discussion the general conception of the paramount importance of the intimal changes although on grounds of probability, weakening of the media has always been sought. The presence of lipid substances in the intima followed by a great hyperplasia of the connective tissue and to a less extent of the elastic tissue is agreed upon. Every conceivable explanation has been offered, based usually on a preformed idea as to what ought to occur. Every form of experiment has been performed in the attempt to reproduce the disease. These resolve themselves into three main groups:

1. Efforts toward affecting the vessel wall by mechanical means through elevation of the blood pressure or otherwise.
2. Efforts toward producing lesions in the vessel wall by various poisons.

## 3. Experiments to show the effects of perverted or one-sided diets.

Of these practically all that fall in the first two groups have failed to produce anything like human arteriosclerosis, while those of the third group have been more successful, especially when combined with mechanical disturbances. Of all the mechanical influences it seems that heightened blood pressure is the only one of any importance but that acting alone it produces little or no change. At most it seems to predispose to the imbibition of excessive lipoids in the intimal layer; whether these lipoids themselves are the cause of the tissue hyperplasia remains to be determined.

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## FAT TRANSPORT IN THE ANIMAL BODY

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The essential constituents of the proteins and carbohydrates—the amino acids and the monosaccharides—are soluble in water, while those of the fats—the higher fatty acids—are not. The amino acids and monosaccharides are, according to our most recent knowledge, carried as such in the blood, while the split products of the fats are built up again into fat for transport. The hydrolysis products of the proteins and carbohydrates pass directly into the blood stream, while it has never been shown that any of the fat goes that way, although it is probable that a small amount does. For these reasons and probably also for others as yet not understood, arrangements are provided in the organism for transporting the fats which are in most respects quite different from those provided for the other foodstuffs. It is proposed in the present discussion to review the available information on this subject.

TRANSPORT ACROSS THE INTESTINAL WALL. In common with all the other food substances the fats are hydrolyzed in the gastro-intestinal canal, the products of the hydrolysis being fatty acids and glycerol. The extent of hydrolysis has been a matter of dispute but ordinarily conditions are such that it is probably nearly, if not quite complete. There is abundance of lipase, mainly from the pancreas but also from the intestinal glands, to hydrolyze many times the amount of fat ordinarily found in the diet. Due to the mechanism for emptying the stomach, the fat is supplied to the intestine in small amounts. There is always some free fatty acid in it when it reaches the intestine since all natural fat contains fatty acid, and the amount is increased by cooking and probably also by the gastric lipase. An abundant supply of alkali is furnished by the various secretions which empty into the intestine—bile, pancreatic and intestinal secretions—and the alkali uniting with the fatty acids forms soaps, by the aid of which the unsaponified fat is emulsified so as to be the more readily hydrolyzable. Added to these factors is the removal of the products of hydrolysis by absorption,

which, since hydrolysis is a reversible reaction, promotes its completeness. In the face of so complete a mechanism for hydrolysis the conclusion is almost inevitable that, whatever be their later fate, the fats are ordinarily completely saponified in the intestine. On the other hand the structure of the absorbing surface of the intestine, the presence there in enormous number of leucocytes which are generally loaded with fat and which are known to pass through the intestinal epithelium, the fact that non-motile bacteria pass across the intestine when fed along with fat, the histological picture of the epithelial cells during fat absorption, which although perhaps better explained by the absorption of fat as split products, does not preclude the absorption of unsplit fat in an ultramicroscopic state of division, together with the fact that only fat is found in the lacteals and thoracic duct, make it unsafe to assume that fat is absorbed wholly in the hydrolyzed form and renders desirable also the careful consideration of the possibility of the passage of unaltered fat across the intestinal wall.

*The histological study of fat absorption.* The most important investigations on this subject are those of Schaefer and Heidenhain who, however, arrive at opposite conclusions as to the way in which the fat passes across the intestinal walls. Recent work by Clark and Clark and by Evans, while not directly concerned with fat absorption from the intestine, is still of great importance in its consideration.

Schaefer (1), in discussing the phenomena occurring in the intestinal epithelium during fat absorption, first noted that there is no definite or at least no rigid cell membrane surrounding the columnar epithelial cells of the villi; that soft bodied cells such as leucocytes occurring between them are able to indent and to work their way through between them. During fat absorption the epithelial cells become filled with fat globules of various sizes, generally largest in the part between the nucleus and the thickened border, and often quite small near the attached end of the cell. Sometimes the greater part of the fat is accumulated in the inner, sometimes in the outer part of the cell, these conditions probably representing the different stages in its absorption. The appearance shown in different preparations is such as to indicate a primary accumulation of fat in the outer or free half of the cell, and its gradual passage down into the inner or attached half, accompanied by the breaking down of the larger particles into smaller ones preliminary to passage out of the cell. The striated outer border (near the lumen) has been the object of a good deal of interest in connection with the passage of fat into the epithelial cells. A few investigators

from Wiedersheim (2) onward claim to have observed what appear to be amoeboid protrusions from the cell border into the intestine and have ascribed to these processes the function of engulfing fat particles from the intestine and transferring them to the interior of the cell. This idea of the method of entry of fat particles into the cells is given sufficient credit by histologists to be included in at least one modern text book (3). However, most investigators have been unable to find even a trace of such outgrowths and the possibility, if present, of their having any function in fat absorption is remote, for the reasons that if they could engulf fat particles they could also take in molecules of starch or protein, which are much smaller, but which are known not to pass the normal intestine; also that the appearance of the fat particles in the cells represents a gradual growth rather than a sudden transference, and that fat globules have never been observed in the substance of the striated border, although such an appearance would be frequent if the globules did pass through this border. Schaefer has found that leucocytes accumulate in the mucous membrane in great numbers during fat absorption and he believes them to be of great importance, if not the main factor in the transference of fat from the epithelial cells to the lymph system. His claim assumes the greater weight in the light of recent work by Clark and Clark (see below). The leucocytes are generally very abundant at the base of the epithelial cells, between them and the basement membrane, but are less abundant between the cells, and occur only occasionally in the intestinal lumen. They are present in large numbers in the interior of the villi and occasionally in the lacteals, especially near the end. That they pass from the villi into the lacteals is shown by the fact that they may be seen partially in and partially outside the lacteal. During fat absorption they are always filled with fat globules, even though the epithelial cells may not be. Schaefer ascribes the main transport of fat from the epithelium to the lymphatics to these cells, and believes that the epithelial cells becoming visibly filled with fat only when the leucocytes fall behind with their work of transport, acting thus as workshops in which the absorbed fat constituents—glycerol and fatty acid or soap—are recombined as fat and stored against the time of removal by the leucocytes. In Schaefer's opinion the leucocytes, after carrying their load of fat into the lacteals, break down, since although they are found in considerable numbers in the lacteals near their beginning, they cannot be found in the thoracic duct. Schaefer's findings and deductions thus provide a complete hypothesis as to the passage of fat from the intestine into

the circulation. Its acceptance is based on the ability of the leucocytes to do the work, which may be questioned, although they undoubtedly are an important factor. From his hypothesis the inference follows that the manner of passage of fat through the two surfaces of the epithelial cell is different—absorption into the cell taking place as fatty acid and glycerol, while passage out into the leucocytes is as finely particulate fat.<sup>1</sup> The transference of fat in a very fine state of division—as an invisible colloidal suspension—has never been considered and is a fruitful possibility, since living cells appear to have the ability not only of building up large globules of fat but also of reducing these again to invisibility.

Heidenhain (5) differs from Schaefer in some important particulars as to his explanation of fat absorption. Commenting on the claims of Zawarykin (6) who believed that the leucocytes alone were concerned with the transfer of fat from the intestine to the lacteals and that the epithelial cells took no part, he admits that the leucocytes undoubtedly can take up fat from the intestine, but believes their part to be secondary for the following reasons: *a*, In newborn puppies when fat absorption is in full progress leucocytes are seldom found in the epithelium, while in fasting they are often present in large numbers. *b*, Leucocytes containing granules stained black with osmic acid are often found in the Lieberkuhn's glands and their presence is difficult to explain since there is no apparent reason why they should transport fat there and the glands themselves do not absorb fat. *c*, Granules staining black with osmic acid may be found in fasting animals near Lieberkuhn's glands and less numerous in the gland itself, and evidence is brought to show that probably some substance other than fat is responsible for the color with osmic acid.

The presence of fat between the epithelial cells observed by some workers (7) Heidenhain is inclined to regard as due to muscular contractions of the villi during fixing, since it cannot be observed in the epithelial cells of animals whose villi have no muscles (frog).

The absorption of unchanged fat through the agency of outgrowths of the epithelial cells of the nature of cilia as claimed by Thanhofer (8) he believes to be very doubtful because of the inability of many histolo-

<sup>1</sup> Regarding the necessity for hydrolysis of the fat before absorption, one reason is probably that of protecting the organism from unsaponifiable fatty materials (4) but once having passed the test of saponification it is very possible that fat may then not have to submit to hydrolysis again but may be passed along in particulate form.

gists (himself included) to demonstrate the presence of anything of the nature of cilia.

For the transfer of fat from the epithelial cells it is his belief that contraction of the protoplasm is responsible as is the case for the transfer of water.

Inside the villi the fat, as relatively coarse globules, moves in the pericellular fluid contents (with the exception of the *small* amount taken up by the leucocytes) and does not assume the dustlike fineness of its final form until it reaches the chyle vessels.

Clark and Clark (9) have recently presented some experiments which are of great interest in connection with the rôle of the leucocytes in fat transport in the intestine and elsewhere, and are in striking support of the claims of Schaefer as regards this function. Drops of fat or fatty acid 30 to 70 $\mu$  in diameter (olive oil, cream, yolk of egg, oleic acid) were injected into the tissue of the tails of tadpoles and the resulting reactions noted. In the case of olive oil, soon after the injection, leucocytes were observed to pass through the walls of the nearby blood vessels and to wander toward the oil droplet. On reaching it they flattened out, formed a ring about it and in a few minutes became pigmented, the pigment being apparently minute particles of oil. Lymph vessels grew out to the oil in from a few hours to two or three days, depending on the distance, and remained in contact with the oil and the leucocytes for several days. No pigmented leucocytes were seen to enter the lymphatic, but the oil droplets in the leucocyte in contact with the lymph vessel became gradually smaller until the cell became clear. Fine, free fat droplets were engulfed by leucocytes, were then reduced in size and replaced by minute pigmented droplets.

In the case of oleic acid, within a minute or two after injection, the clear globule became opaque and granular—brown by transmitted light. The leucocytes responded more quickly and in larger numbers than with olive oil (irritation by the fatty acids?) forming a ring several layers deep and soon becoming deeply pigmented. The lymph vessels responded as with the olive oil and a study of the leucocytes showed that they were continually moving away from the fatty acid, wandering up to a nearby lymphatic and in fifteen minutes to one half-hour moving away, having lost their brown pigment. None were observed to enter the lymph vessel. Absorption of oleic acid or sodium oleate was more rapid than olive oil.

When cream or yolk of egg (finely divided, emulsified fat) was injected, the same reactions were observed—the leucocytes acting as

carriers but working in this case more rapidly. When injection was near a lymph capillary, loaded leucocytes came into contact with the lymphatic within three hours after the injection. If, however, the leucocytes had far to go they lost their pigment (and therefore their load of fat?) before reaching the lymph vessel, which however continued to grow in their direction and that of the fat mass, indicating perhaps a diffusion of soluble and therefore invisible products (hydrolysis products?) from the leucocyte. The absorption of cream or yolk of egg was so rapid that there was often no time for the lymphatic to grow out to the injected material. Leucocytes and lymphatics were the only structures reacting to the injected fat, the blood capillaries, if anything, growing away from it. These reactions were limited to fat and fatty acid. No reaction could be obtained with fat-like substances such as mineral oil.

The work of Clark and Clark indicates that the leucocytes can take up unsplit fat and transport it fairly rapidly, and their presence in the intestine in such large numbers during fat absorption and the fact that they are always loaded with fat even when the epithelial cells contain very little, indicates that they may have a very considerable part in certain stages of the absorption, i.e., transport to the lacteals. But numerous as they are in the lacteals and around the epithelial cells they rarely get out into the lumen of the intestine, so that the first stages at least of the absorption of fat must be referred to the epithelial cells themselves. The possibility that the epithelial cells act phagocytically, engulfing the unchanged fat particles, seems doubtful from the fact that fat particles have never been observed in the cuticular membrane, and that most of the considerable mass of evidence available points to the absorption of fat in the form of its hydrolytic products—glycerol and fatty acids (or soaps).

*Form in which fat leaves the intestine.* The experimental data regarding the form in which fat passes out of the intestine may be considered under four headings; first, that bearing on the degree of hydrolysis of fat in the intestine; second, that regarding the absorbability of the split products; third, the behavior of the intestine toward substances of a fatty nature which are not fat; and fourth, the evidence regarding the absorption of unchanged fat.

1. *The extent of fat hydrolysis in the intestine.* No matter how much fat is passing along the intestine or how little is being absorbed, the fatty material found in the lower intestine and the feces consists almost entirely of fatty acids (or soaps) (10), which indicates clearly that ample

facilities are provided for hydrolysis. When absorption is taking place with consequent removal of the products, hydrolysis must be even more complete, since it is a reversible process and follows the law of mass action. In healthy animals enough pancreatic lipase is provided to split many times the amount of fat ordinarily present in the food. Added to this main source of lipase is that present in the gastric secretion (11) which, although not ordinarily important because of its sensitiveness to acid, may bring about considerable hydrolysis when the gastric acidity is low and especially when fat is present in an emulsified form (milk, yolk of egg, etc.). In addition to the gastric lipase some is supplied in the intestinal secretions (12), which is probably responsible for the splitting of fat which is found to take place in the absence of the pancreatic secretion.

Owing to the fact that the lipases are soluble in watery mixtures but not in fat, their hydrolytic action can be exerted only at the fat-water interfaces, and unless the fat surface thus exposed is great, splitting will be slow. Hence the importance of emulsification, which by reducing the size of the fat globules increases their surface. Abundant provision is made for emulsification in the intestine. The most important emulsifying agent is undoubtedly soap, the fatty acid for the formation of which is present to a small extent in all natural fats and the amount is increased by cooking and by the gastric lipase. Alkali is supplied by the secretions entering the intestine—pancreatic juice, bile and the various intestinal juices which are all more or less alkaline. The fat containing fatty acid ordinarily enters the intestine in small portions, is met by the alkaline secretions which unite with the fatty acid to form soap and by the motion of the intestine the unsplit fat is well emulsified. Additional emulsifying agents or stabilizers—proteins, lecithin, etc., are furnished by the secretions, and even though, as has been noted several times, the reaction of the intestine is slightly acid throughout its length (13), the acidity is due mainly to carbonic acid, which has little effect on a soap emulsion in the presence of bile and pancreatic secretion (14). The fact that it is not always possible to find an emulsion in the intestine during fat absorption (15) is not a serious objection since such a finding would be made if hydrolysis were rapid in relation to the amount of fat present—all the fat present being in the form of soap.

2. *The absorbability of the split products of fat—fatty acids or soaps and glycerol.* That the split products of fat were absorbable was demonstrated very early in the study of the behavior of fat in the intestine.

Radziejewski (16) showed that alkali soaps were absorbed; Perewoznikoff (17) showed that a mixture of alkali soap and glycerol was absorbed and synthesized into fat, the lacteals having the usual appearance after a fat meal and the epithelial cells containing fat globules. All the more recent evidence confirms the earlier findings (18) and the fact seems proven that the intestine can absorb fatty acids or soaps and glycerol and synthesize fat from them.

3. *The behavior of the intestine toward substances of a fatty nature other than ordinary fat.* A number of workers have experimented with substances of this type in the hope that thereby information regarding the manner of absorption of fat might be obtained, and the results have been quite illuminating. *a.* Substances which are hydrolyzable in the intestine—ethyl esters of the fatty acids (19), amyl esters of the fatty acids (18), optically active mannite esters of the fatty acids (20). These are all completely hydrolyzed in the intestine and the fatty acid component appears in the chyle as the triglyceride showing that a synthesis with glycerol has taken place. *b.* Non-hydrolyzable substances which by one means or another can be emulsified. Two substances have been used for this purpose—esters of the fatty acids with cholesterol or related substances which readily yield a fine emulsion with water, and the paraffin hydrocarbons which are soluble in fat and which can be emulsified with it. Absorption of these substances has been tested in two ways—by determinations of unabsorbed residue in the feces (21) and by determining the absorbed substance in the chyle (22). In neither case was there any evidence of absorption. A similar rejection of paraffin hydrocarbons was observed by Clark and Clark in their study of the absorption of fat and fat-like substances injected into the tails of tadpoles.

4. *Absorption of unhydrolyzed fat.* There is no good evidence that fats can be absorbed unchanged in any considerable amount. The belief that such was the case was based largely on the observation that the appearance of the fat in the intestine and the lacteals was the same—a milky emulsion. The objection, that the particles of suspended fat in the lacteals were much smaller than those in the intestine has never been explained away but a reasonable answer would be that only the finer particles of the fat of the intestine were absorbed, and that one of the purposes of intestinal action was to reduce the fat particles to absorbable size, the processes of hydrolysis and emulsification being directed to that end. Hydrolysis of fat by the lipases in the intestine was never denied by the supporters of the theory that fat was

absorbed as such in finely divided form but their claim was that only enough hydrolysis took place to produce soap for emulsification. The repeated statements of the histologist that no fat particles can be observed in the cuticular membrane which is the first line of action of the epithelial cells have never been answered and could be explained only by the assumption that the fat particles were so fine as to be ultramicroscopic which, so far as is known, has never been made. There appears to be nothing inherently impossible in the reduction of fat particles to ultramicroscopic size since fat is known to occur in such a (colloidal) condition in nature as stored material in egg yolk and in many cells, both plant and animal, and as noted above, the reduction in size of fat particles to invisibility has been observed in leucocytes and in intestinal epithelial cells. But the ability to reduce fat to particles of ultramicroscopic size appears to be confined to the living interior of cells and could probably not take place in the intestine. Even if it were possible to reduce fat to the colloidal condition in the intestine a special mechanism must still be assumed for its absorption since other substances in colloidal suspension such as proteins and starches and even relatively simple substances in true solution such as the various disaccharides are not allowed to pass into the blood unchanged. Other evidence of absorption of unchanged fat such as the absorption of colors which are soluble in fat but not in water, and the deposition in the fat stores under certain conditions of large amounts of fat which is chemically the same as the fat of the food can be equally well explained by the hydrolysis-synthesis theory, since the fat dyes used were shown to be soluble in fatty acids (23), and since the fat found in the lacteals and hence in the blood and fat stores must be built up largely from the available hydrolysis products of the fat in the intestine.

To sum up: Abundant facilities are provided for the hydrolysis of those esters of the fatty acids which hydrolyze with the same or greater ease than the fats. The split products are readily absorbed and converted into fat in the passage through the intestinal wall. Substances which cannot be hydrolyzed and so rendered water-soluble are not absorbed no matter in what form they may be presented. The prevailing belief that fats are completely hydrolyzed in the intestine and absorbed as the hydrolysis products thus has most of the evidence in its favor, although the possibility of the absorption of some unchanged fat cannot be absolutely excluded. The reason for the hydrolysis, which appears to be universal for all food substances, is not far to seek. On the one hand there is the necessity for the exclusion of substances which

as presented would be useless and harmful to the organism, such as unchanged proteins, complex carbohydrates or fat-like substances other than fat; and on the other, since no food substance is presented in immediately usable form, more or less complete hydrolysis must take place in any case, if not in the intestine then in the tissues. The two purposes are combined in the intestine, which by means of the same process rejects harmful or useless substances and reduces the useful material to fragments which can be used at once by the tissue cells which require them. There is therefore no need to assume a special mechanism for the transfer of fat from the intestinal lumen into the epithelial cells—this takes place according to the usual rule by hydrolysis, which is as usual complete to the limits of hydrolysis for the individual substance, followed by absorption of the split products in water solution. (Attention should be called to the fact, which is perhaps not ordinarily sufficiently appreciated, that hydrolysis in the intestine is carried to the utmost limit; any further change such as division of the dextrose molecule, deamination of the amino acids or breaking of the fatty acid chain would involve other changes than simple hydrolysis.)

*Passage of fat out of the epithelial cells.* The fat fragments after reaching the interior of the epithelial cell appear to be built up into fat again and retained in the cells for some time—at least this is the picture when much fat is being absorbed. When little absorption is taking place no such accumulation can be observed, but the leucocytes, according to Schaefer's observations, are always full of fat whether much or little is being absorbed, and his belief is that the function of the epithelial cells is to receive the hydrolysis products, synthesize them and store the fat until the leucocytes can transport it to the lacteals. Both Schaefer and Heidenhain agree that the fat from the epithelial cells is transferred to the lacteals without alteration other than changes in size of particles, Heidenhain believing that the particles are expelled into the body of the villus by contractions of the epithelial cell protoplasm, while Schaefer thinks that they are transferred to the leucocytes by contact and by them to the lacteals.

In contrast to these opinions based on histological observations, Loevenhart (24), on the basis of chemical evidence, believes that the passage out of the cell, like the passage into it, is accomplished by a hydrolysis and synthesis, and also that wherever in its subsequent history the fat has to pass a cell wall the same hydrolysis and synthesis take place. His assumption requires the presence at all these points of a sufficient supply of lipase to bring about these changes in the time

ordinarily consumed in transferring the fat from the intestine to the tissues and he brings evidence to show that lipase is present in all tissues, and especially in those which are ordinarily most concerned in fat metabolism—the liver, active mammary gland, blood, lymph and intestinal mucosa. He notes particularly its presence in those places where fat synthesis is known to take place—the active mammary gland and the subcutaneous fatty tissue. The test employed by him to show the presence of lipase is the ability of a water extract of the tissue to hydrolyze ethyl butyrate. Aside from the fact that the splitting obtained by him is rarely very extensive, being ordinarily less than 5 per cent in forty hours and therefore possibly unimportant as a factor in fat metabolism, his experiments have aroused critical comment in several quarters. Regarding the use of ethyl butyrate as a test of the presence of lipase, it was pointed out by Arthus (25) and later by Jansen (26) that a test carried out on the esters of the lower fatty acids such as monobutyryl (and therefore also ethyl butyrate) cannot give a true measure of lipase action since these esters are much more readily hydrolyzed than the triglycerides of the higher fatty acids and regarding which Loevenhart himself gives a reference to Euler (27) to the effect that the hydrolysis of an ester is greater the stronger the constituent acid; therefore the higher fatty acids being relatively much weaker than butyric acid their esters would be much more slowly hydrolyzed than those of butyric acid.

Bradley (28), using the same technique as Loevenhart, was unable to corroborate his findings in some important particulars. He found no broad correlation between fat and lipase content of tissues. Some of the most active fat-producing tissues (mammary gland) are relatively poorer in lipase than others which never normally contain or produce more than a small percentage of fat (lung, kidney, muscle). He concludes that quantitative comparison of fat and lipase in animal tissue gives no positive evidence in support of the theory of enzyme synthesis.

Thiele (29) found that blood and chyle contain a ferment which can hydrolyze phosphatides but not fat, and also (30) that tissue extracts (excluding pancreatic extract) similarly cannot hydrolyze fat but can hydrolyze the phosphatides.

Porter (31) found ferments capable of splitting ordinary fat (olein, stearin) present in most tissues but generally in the merest traces, except in the case of the pancreas, the amount of splitting taking place being so small as to be of no practical significance in a consideration of fat metabolism. Ferments capable of splitting lecithin were present in larger amounts.

In earlier work Kastle and Loevenhart (32) found that pancreatic extract was less active on ethyl butyrate than liver extract but the reverse was the case when fats were used as zymolytes.

It appears desirable therefore to differentiate between enzymes which hydrolyze the fats readily—the true lipases—and those which work slowly on the fats but readily on the esters of the lower fatty acids and on lecithin and which may be called esterases. Lipases appear to be present in significant amounts only in the intestinal secretions of the pancreas and other intestinal glands, while the esterases are of quite general distribution. In view of the importance of lecithin as an intermediate stage in fat metabolism the esterases are therefore to be regarded as potentially of considerable significance. Since fat is synthesized in the intestinal epithelium, the presence there of a true lipase must, however, be admitted and the question of the passage of fat out of these cells into the villi or into the leucocytes by the hydrolysis-synthesis method must be left open.

*The bile in fat absorption.* Along with the abundant lipase supplied to the intestine there is secreted a similarly large volume of bile, the importance of which in fat absorption has been known for a long time. Although it furnishes no lipase, its exclusion from the intestine results in as much or greater loss of fat than the loss of the pancreatic secretion. Its main function appears to be in increasing the solubility of the fatty acids and soaps in the intestine and so aiding their transport through the absorbing cells (33). It also increases the activity of the lipases (34), increases intestinal peristalsis and provides materials which aid in providing optimum conditions for fat digestion. These later are alkali (carbonate and bicarbonate) to be used in the formation of soaps and in preserving the reaction of the intestinal fluids, and lecithin, mucin, etc., which stabilize the fat emulsions.

Considering all the evidence together, there is every reason to believe that the fat is completely hydrolyzed into glycerol and fatty acids before it passes from the intestine. The split products are taken up by the epithelial cells and converted by them into fat which, depending on the rate of inflow from the intestine, is either passed on at once to the lacteals or stored temporarily. As to what happens to the fat from this time on to its appearance in the thoracic duct very little is known with certainty beyond the fact that before passing out of the epithelial cells the fat particles are reduced to invisibility. Whether this means re-hydrolysis or whether the division is a physical one is not clear, but since fat is synthesized in these cells there must be lipase present and

re-hydrolysis is at least a possibility. Heidenhain's claim that the fat particles are expelled from the epithelial cells by contraction of the protoplasm is difficult to accept since the cells are fixed at the basal end and there is no more reason to assume an amoeboid character at this end than at the other. The leucocytes, in view of the work of Schaefer and later of Clark and Clark, undoubtedly do carry fat to the lacteals, but whether their function is of importance in intestinal absorption remains to be shown, the whole question depending on whether there are enough of them to do the work. As shown by Clark and Clark, the leucocytes have the power, like the epithelial cells, of building up large fat globules in their bodies and also of reducing them again to invisible size, but whether this involves a double hydrolysis and synthesis also remains to be proven, since lipase in significant amounts has not been demonstrated. Owing to the absence of lipase in amount sufficient to bring about these numerous hydrolyses and syntheses in the various tissues it appears better for the present to assume that the primary hydrolysis in the intestine and synthesis in the epithelial cells is the only one the fats undergo and that all further transport across cell walls is either as particulate matter in a fine state of division or by transformation into water-soluble substances of the nature of lecithin, for the hydrolysis (and therefore synthesis) of which enzymes are available. As will be seen later, both methods are probably employed.

*Passage of fat into the circulation.* Fat absorption is generally discussed only in connection with the passage of fat into the lacteals and the thoracic duct and so to the circulation indirectly since this seems to be the main and, up to the present, the only demonstrable path of absorption. What are the indications that fat may pass into the circulation in other and possibly more direct ways? It has never been possible to recover all the absorbed fat from the chyle of the thoracic duct, in fact about 60 per cent appears to be the maximum recoverable (35),—which would indicate either that some of the absorbed fat had been stored somewhere along the lymph tract or that some had been absorbed directly into the blood stream. Evidence regarding absorption directly into the blood stream from the intestine is contradictory. Bornstein, under Heidenhain's direction (36), compared the fat content of the carotid and portal blood at the height of fat absorption and found the fat content of the carotid artery to be slightly higher both in whole blood and in the dry residue than in the portal vein. D'Errieco (37) found that during normal fat absorption the total solids and the fat content of the portal vein were always higher than those of the jugular.

After ligation of the thoracic duct the fat content of the dry residue of the portal vein was still slightly higher than that of the jugular, but nothing like as high as would be expected. D'Errico's experiments may be criticised from the fact that samples were not taken simultaneously but from one-half to one hour apart, and that absorption of all kinds from the intestine was interfered with by the operation since the total solids in the portal vein diminishes considerably. These experiments have been repeated recently by Zucker (38), with negative results. He ascribes d'Errico's positive results to faulty technique and concludes that no marked participation of the blood vessels in fat absorption can be assumed. It seems therefore that although the possibility of absorption of fat in small amounts by the blood vessels cannot be denied, there is no evidence yet available to prove it. It should be noted however that none of the methods used for the determination of fat can be relied on to give results accurate to within much less than 5 per cent of the true value, and in view of the extensive circulation through the portal system a difference of 2 or 3 per cent would account for a considerable absorption. It is perhaps significant in this connection that Clark and Clark found no attraction between blood capillaries and fat or leucocytes such as is exhibited between them and lymph capillaries. Excluding direct absorption into the blood the 30 to 40 per cent of absorbed fat which cannot be collected from the thoracic duct may be accounted for either by storage in some place along the lymph path or by entry into the lymph or blood systems by other channels than the thoracic duct. No such connection has ever been demonstrated and if present it is probably of little importance since when the thoracic duct is tied off little or no fat reaches the blood (37), (39).<sup>2</sup> As to the possibility of fat storage along the path of transport, fat is present in the epithelial cells and the intestinal leucocytes for some time after it has ceased to be absorbed from the intestine and it has been found in quite large amounts in the leucocytes in the lymphoid tissue of the intestine (Heidenhain, p. 85) even during fasting. Nevertheless the amount that could be stored in this way is probably not great, and in spite of the lack of evidence of direct absorption into the blood stream the probability is that some at least of the fat is absorbed in that way. The

<sup>2</sup> Too much weight should not be placed on results obtained by tying off the thoracic duct, for the shock caused by the operation and by the backing up of the chyle must be great, and the failure to demonstrate differences in fat content of the blood during absorption may indicate only that the fat is being removed from the blood as fast as it enters.

direct evidence presented by Bornstein and Zucker against absorption into the portal system may mean only that their methods of measurement were not sensitive enough.

TRANSPORT FROM THE BLOOD TO THE TISSUES: *Changes in blood lipoids during fat absorption.* The larger and only traceable part of the fat from the intestine is delivered into the blood stream from the thoracic duct in the form of suspended particles of finely divided pure fat. Traces of soaps or fatty acids have been reported but these are little if any more than could be accounted for by the methods of separation employed. The finely suspended fat in the blood is, as far as can be determined, exactly the same as that in the chyle—particles of about  $1\mu$  in diameter, vary even as to size and having a pronounced Brownian movement. During the time that this fat is in the blood and excepting only when little fat is being absorbed, there is to be noted a considerable increase in phospholipoid—"lecithin"—and sometimes also of cholesterol. The increase of lecithin is reported by all investigators who have dealt with the lipid changes in the blood during fat absorption (40). As far as has been determined, increases in lecithin are most marked in the corpuscles and since fat was increased in them also the inference seems justified that fat is being taken up by the corpuscles and transformed into lecithin (40). The fact that further (unpublished) experiments indicate that the increase in the corpuscles does not always take place need not invalidate the assumption since from work on persistent lipemia (41) it is probable that the lecithin soon passes from the corpuscles to the plasma. As regards cholesterol, while most investigators have not found any increase during fat absorption (38), (40), (42), others (43) have noted increases. The differences in findings as regards cholesterol may be due to the fact noted by Iscovesco that changes in cholesterol values generally do not come until late in the period of absorption and so may be missed if the observations have not been continued long enough. Undoubtedly also, when small amounts of fat are being absorbed no changes at all may be noted in cholesterol. Allowing that the increase of cholesterol in fat absorption is a normal event there appears to be a definite sequence in the lipid changes following the appearance of large amounts of fat in the blood—the lecithin increasing first and then the cholesterol. It is probable that the increase of fat must reach a certain magnitude before lecithin is formed in notable amounts and that lecithin must be similarly increased before cholesterol begins to increase. In persistent lipemia both lecithin and cholesterol are increased along with the fat, the

cholesterol to a greater extent than the lecithin (41). In cases of persistent lipemia that have been studied from their beginning throughout their course (41) the same sequence in the appearance of fat, lecithin and cholesterol noted above may generally be observed, fat increasing first, then lecithin and finally cholesterol. At the subsidence of the lipemia, lecithin and cholesterol persist after the fat has gone and of these cholesterol persists longest. Since lecithin is almost certainly a factor if not a stage in fat metabolism its increase following increase of fat is readily explainable, but it is more difficult to account for the increases in cholesterol, since although it does to some extent (about one-third of its amount) form esters with the fatty acids, these are more difficultly saponifiable than the fats (while lecithin is more readily saponifiable) and can for that reason hardly be regarded as important in fat metabolism. Cholesterol and lecithin are known to be antagonistic in many of their relations in the living body and it may be that the increase of cholesterol following that of lecithin is a reaction whose purpose is to offset the injurious effects of an abnormally high concentration of lecithin.

Further evidence that lecithin (phospholipoid) may be of great if not of supreme importance in the transport of fat in and from the blood is provided by the work of Meigs and his co-workers (44) on milk-fat secretion in cows. These investigators found that during milk secretion the difference in the lipoid phosphorus values of the blood plasma before and after passing through the mammary gland is sufficient to provide the *entire* amount of fat secreted in the milk. Allowing for considerable errors in the calculations on which this assumption is based, the fact remains that a large proportion of the milk fat probably has its origin in the phospholipoid of the blood. There is considerable evidence of an indirect nature which may be brought to the support of the thesis that lecithin in the blood is the main form of transport of fat, and the precursor of fat in the tissues, and conversely, that fat in the tissues on entrance into the blood is changed to lecithin. While, as discussed above, it is improbable that lipases are present in the tissues in amounts sufficient to bring about the passage of fat to and from the cells by hydrolysis, all workers are agreed that there is abundant enzyme in the tissues for the transformation of lecithin (esterases, lecithinases). Lecithin is present in relatively large amount in the organ which is known to be most active in fat metabolism, e.g., the liver; also in the heart where a large reserve supply of readily available energy is required, and in the brain where, although the energy exchange

is not great, the conditions for the energy transformation must be very exactly adjusted. Lecithin is the only compound of the fatty acids (with the exception of the soluble soaps which are known to be toxic) which is miscible in water and which can therefore readily follow the lines of water transport. These, together with the observation that lecithin is formed in the blood during fat absorption, present a strong argument for the belief that it is the main form in which fat is moved in the animal body.

On the other hand it cannot be denied that fat may be removed from the blood, at least temporarily, in other forms than as lecithin. During fat absorption, and at other times of large fat transport, as in fasting, diabetic lipemia and in phosphorus and other poisoning, the liver is found loaded with fat, mainly as such although the lecithin content of the liver, if not greater at the time, is generally soon increased (45). Fine emulsions of colored or otherwise marked fat when injected into the circulation are found to collect in certain definite places—the liver, bone marrow, spleen and muscles in the order named (46), in which respect the fat particles behave in the same manner as particles of other foreign matter. For this reason a brief review of the factors involved in the removal of finely particulate matter from the blood is desirable. Evans (47) has called attention to a group of endothelial cells, called by him macrophages, which are found in the vascular system at just those places mentioned above where injected fat is found to have collected, i.e., capillaries of the hepatic lobules, capillaries and venules of the spleen, capillaries and venules of the bone marrow, capillaries and venules of the hemal glands, lymphatic sinuses of the lymphatic glands. Other macrophages which are more or less fixed include reticulum cells of lymph glands and similar cells in the splenic pulp and bone marrow which are probably endothelial; also cells not directly related to the endothelium designated variously as clasmocytes or wandering cells. Besides these there are free macrophages—mononuclear cells found in the serous cavities, in the lymphatic sinuses of lymph glands, in the splenic and hepatic cavities but rarely observed in the peripheral blood stream. Recently Simpson (48) in Evans' laboratory has studied these cells experimentally and has outlined conditions under which vast numbers of these macrophages may be set free in certain portions of the circulation. A characteristic feature of the macrophages is the presence of abundant, various sized and often delicate pseudopodia which may cover their surface and which would indicate at once their function in engulfing whatever free particles were

attracted to them. The phagocytic nature of many of these groups of cells has long been known and it is easy to demonstrate with certain dyes, colloidal metals, etc. The actual demonstration of the phagocytic activity of these cells toward the finely suspended fat present in the blood during fat absorption has never been made, but from the general observation of Bondi and Neumann (see above) that injected fat may be found in just those places where these cells are known to be abundant, gives a strong indication that they are concerned in the removal of suspended fat from the blood. That lipoids accumulate in these cells has also been known for some time (49), and the parallelism between the distribution of the vital dyes and the fat bodies has been pointed out. Also it is precisely these cells filled with anisotropic lipoids (cholesterol esters) which constitute the xanthoma and xanthelasma cells and it is especially significant that animals fed for a long time on cholesterol suffer a very general conversion of their wandering cells into xanthoma cells (Evans, p. 257). Whether the fat so taken up is passed on from these cells into the tissues or whether it is stored there temporarily to be given up to the blood stream later for transformation into lecithin cannot be surmised, but it is known that dye granules may remain in these cells for a long time.

**PASSAGE FROM THE TISSUES:** *Fat in the tissues.* Lipoid material exists in the tissues in several forms, of which fat, phospholipoid (lecithin, etc.), cholesterol and cholesterol esters are present in largest amounts and are best known. The phospholipoid has been found to be characteristic of the organ, e.g., always more abundant in the kidney or liver than in the muscles. In different animals of the same species it varies relatively little and although the variation is somewhat greater in different species the values are quite constant. The content does not vary with alimentation (50). A characteristic relation between cholesterol and the fatty acid content of most tissues has also been noted (51). Inanition produces a rise (which may be only relative) in cholesterol in the tissues, especially the muscle and liver (52), and in the lipid phosphorus (53) especially in the lungs. The fatty acid content diminishes in inanition and the relation  $\frac{\text{Fatty acid}}{\text{Lipoid phosphorus}}$  is much below normal and much lower than in any phospholipoid so far isolated, indicating that some of the lecithin present may contain only one fatty acid group instead of two as normally.

The main form in which lipoid material is stored in the organism is however as fat and although there are but few locations in the organ-

ism where traces of fat may not be found it is in general laid away in a special tissue—the adipose tissue—which is collected characteristically in definite locations (54). There is a considerable layer under the skin—the panniculus adiposus, around the internal parts—the kidney, filling up furrows in the heart—in various situations beneath the serous coats or between their folds as in the mesentery or omentum, around the joints, in large amounts in the bone marrow, often in large amounts between the muscle fibers and occasionally in the muscle fibers themselves. The adipose tissue always has a copious blood supply and the lymphatics of the fat tissue are in close relation to the blood vessels. The adipose tissue is modified connective tissue which apparently is predestined to act as a fat store from the early stages of development (55). Other portions of connective tissue may be pressed into service as required, developing a special blood supply for the occasion and passing back to ordinary connective tissue again when it gives up its fat, which is not the case with true adipose tissue.

Fat may be deposited in many cells other than the adipose tissue—cartilage, liver (especially during fat absorption from the intestine), epithelium and in the muscle fibers. In many of these locations the fat deposits are independent of the nutritional condition of the body whereas those in the adipose tissue vary with it. Greene has made a study of the storage of fat in the Pacific Coast salmon at various times in its migration from the sea to the spawning grounds and his data are of special interest in connection with the storage of fat in muscle tissue (56). In the dark muscle, fat is stored chiefly within the fibers but to some extent between them. In the pink muscle (the great lateral muscle of the body) fat is stored wholly between the fibers. In the constantly active fin muscles there is only a small amount of stored fat which is chiefly between the muscles. After feeding ceases the fat slowly disappears but is never wholly consumed although greatly reduced at the time of death.

The liver is generally regarded as a temporary fat storehouse and there are many observations on record to show that fat accumulates in this organ under a great variety of conditions. Thus during fat absorption (57) the fat of the liver is increased. In the course of poisoning with many substances, e.g., phosphorus, the liver generally becomes loaded with fat. Delayed chloroform poisoning is accompanied by a fatty liver. The condition occurs in chronic alcoholism (58), often in fasting (60) and in diabetic lipemia (59). The frequency with which the liver becomes loaded with fat under such diverse condi-

tions has led Leathes (61) and Hartley (62) to the belief that the liver is an important temporary storehouse for the fat passing out from the tissues and they bring evidence to show that changes preparatory to combustion (desaturation of the fatty acids, combination into lecithin) are brought about there. All workers are not in agreement with them in the belief that the liver is a fat storehouse. Thus Terroine and Weill (63) report that the remarkable constancy of the fatty acid content of the liver and the difficulty of diminishing the content by inanition or of increasing it by hyperalimentation leads them to the belief that the liver is not a storehouse of fat. In a study of the fat changes in the liver in geese under forced feeding, Mayer and others (64) found that it was only in the later stages of feeding when the other fat depôts are full that the liver and blood load up with fat. They conclude that there is nothing in their experiments to show that the liver acts normally as a fat storehouse. Terroine (52) found that the liver preserves its fatty acid content after several weeks of fasting. In hyperalimentation it does not increase except in immature animals. Bell (55), in studies on fat cattle, found fat in the hepatic cells of two moderately fat animals while none was found in the very fat animals. He concludes that there is no relation between the presence of fat in the liver and the nutritive condition of the animal.

Fat is laid down in the cells of the adipose tissue of animals in the same way as has been noted for the epithelial cells of the intestine—appearing first as fine droplets which gradually becoming larger, fuse with each other until the cell is filled with a single large globule of fat. In plant cells which store fat, droplets are never seen (65). The fat is so finely divided and so intimately mixed with the other constituents of the protoplasm that individual droplets cannot be distinguished even with the highest powers of the microscope, and only on germination visible fat droplets are found. It is as though the fat has been present in colloidal form and that flocking out took place only as a result of the swelling produced by germination. This condition is interesting because it is never known to occur in storage cells of animals.

In the laying down of fat in the adipose tissue the cells adjacent to the blood vessels are filled first and the filling extends outwards in all directions, which has also been found to be the case with the liver (55). As to what elements or constituents of the cells are concerned with the laying down of fat, very little is known. Certain granules (Altmann's granules) (66) are always found in cells (55) (intestinal epithelial, adipose tissue, etc.) which are concerned with the storage of fat but too

little is definitely known regarding them to warrant extended discussion at this time. The possible rôle of a lipase or esterase in the transfer of fat in and out of these cells has been discussed earlier in the paper.

When the stored fat is required by the organism it is removed from the adipose tissue first and the removal continues until these cells are practically empty, while even in death by starvation the fat content of other tissues may not be greatly changed (67), (52), indicating that the supply of fat in these cells if used at all is being continuously replenished from the blood. Thus Greene (56) found that the fat inside the muscle fibers of the migrating (fasting) salmon is present in all specimens at all stages of the migration and its quantity is remarkably uniform. At the time of death considerable quantities are still present in the smallest fibers. This fat is not observable in these muscle fibers when the animal is feeding but appears only when feeding stops.

As regards the stimulus to the outflow of fat from the stores very little is known other than that it is obviously the result of inanition of the tissues caused either by direct deprivation of food or by poisoning. As to the immediate stimulus, the disappearance of glycogen from the liver (and other tissues) has been suggested (68) and an antagonism between glycogen and fat in the tissues has been suspected. Extensive bleeding has been found to cause an outflow of fat from the tissues of certain animals (41) but this again may be the result of inanition due to anemia.

*Effect of transport of fat on the blood lipoids.* The outflow of fat from the tissues into the blood may or may not cause an increase in the blood lipoids depending on the balance between the inflow from the stores and the outflow to the tissues in which fat is being utilized. If inflow and outflow are equal, the blood lipid level remains constant. If the inflow is greater than the outflow, due either to flooding the organism with fat beyond its capacity to deal with it as in the lipemia produced by bleeding and also that occurring in the early days of fasting, or to diminished power of the tissues to remove fat from the blood as in diabetic lipemia (41), then the blood lipoids increase until sometimes a visible milkeness (lipemia) is produced. There may however be considerable increases in the lipoids before there is visible lipemia and it cannot be assumed that the increases in this case are due to "soluble" lipoids such as lecithin alone, since cholesterol and fat may both be increased. The form of occurrence of the latter substances then becomes a matter of interesting speculation.

That there is a tendency toward constant values for the lipoids of the blood both in plasma and especially in the corpuscles in the normal animal seems now to be pretty well established (69), and whenever the amount of fat shifted is not too great the blood preserves its constancy of values. After ingestion of considerable amounts of fat the lipid level and balance is disturbed as noted above. In fasting it may or may not be (70), depending apparently on the nutritional condition of the subject (71), or rather perhaps on the availability of the stored fat, since it is apparently the case that fat may be more or less loosely stored, with the result that the stimulus of hunger may produce an excessive or merely adequate outflow depending on the nature of the storage. It is significant that the increases in blood lipoids in fasting take place only in the first days, and after that the lipid content remains constant or slowly diminishes till the death of the animal (Daddi, Terroine). Similarly those substances which cause a transfer of fat from the fat stores via the blood such as narcotics, phosphorus, etc., may or may not cause an increase of blood lipoids, probably for the same reasons. Great hemorrhage in certain animals (rabbits) produces an outflow of fat into the blood far greater than the normal mechanism can take care of, and the result is a lipemia. In other animals (dogs) it is apparently impossible to produce a lipemia by hemorrhage, probably for the reason that in these animals the ability to handle fat is much greater than in rabbits, which are not accustomed to much fat in their diet (41). In all cases examined of lipemia lasting for more than a day or two, not only the fat but also lecithin and cholesterol are much above normal.

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## THE WATER BALANCE OF THE BODY

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Water balance may be defined as the daily relation between the total amount of water entering the organism through the ingestion of liquids and food and the total output of water lost from the body by way of the kidneys, bowels, lungs, and skin. In the intake must be included the water of oxidation.

For the maintenance of health the intake must be sufficient to maintain the amount of water in the body tissues necessary for maximal efficiency in metabolism and in the execution of other physiologic processes. This involves the removal of waste products and the dissipation of heat. Since heat dissipation constitutes one of the important factors governing the measurement of water in the organism, loss of water by the skin and respiratory tract will be discussed in considerable detail. Although the loss of water by the skin and lungs, on the one hand, and by the kidney on the other, are to a certain extent inversely proportional, the function the water serves in excretion by these channels is different and can not be interchanged vicariously.

Water is essential to the life and function of every living cell. While its importance is and always has been known, its unique properties are not, and have not been so clearly recognized.

In his recent book, Henderson attempts to give to water due recognition. He says:

In physics, in chemistry, in geology, in meteorology, and in biology nothing else threatens its preëminence. The physicist has, perforce, chosen it to define his standards of density, of heat capacity, and so forth, and as a means to obtain fixed points in thermometry. The chemist has often been almost exclusively concerned with reactions which take place in aqueous solution, and the unique chemical properties of water are of fundamental significance in most of the departments of his science. . . . The action of water now appears to be far the most momentous factor in geological evolution. The meteorologist perceives that the incomparable mobility of water, which depends upon its peculiar physical properties and upon its existence in vast quantities in all three states of solid, liquid, and gas, is the chief factor among the properties of matter to determine the nature of the phenomena which he studies; and the physiologist has found

that water is invariably the principal constituent of active living organisms. Water is ingested in greater amounts than all other substances combined and it is no less the chief excretion. It is the vehicle of the principal foods and excretory products for most of these are dissolved as they enter or leave the body.

In closing the chapter on water, Henderson goes on to say:

Water, of its very nature, as it occurs automatically in the processes of cosmic evolution, is fit, with a fitness no less marvelous and varied than that fitness of the organism which has been won by the process of adaptation in the course of organic evolution. If doubts remain, let a search be made for any other substance which, however slightly, can claim to rival water as the *milieu* of simple organisms; as the *milieu intérieur* of all living things, or in any other of the countless physiological functions which it performs either automatically or as a result of adaptation.

Water constitutes more than 70 per cent of protoplasm, the structural basis of organic life. Hence it follows that water is of the greatest significance to life. Indeed by some physical chemists, protoplasm is looked upon as essentially an aqueous solution in which are spread out colloidal substances of the greatest complexity. Although we are accustomed to look upon the cell as the seat of metabolism, it is not so clearly recognized that water constitutes the medium in which the chemical changes of metabolism occur, that as a *milieu intérieur* it is essential to life, and that it is fundamental to practically all physiologic processes.

Rubner early called attention to the fact that in starvation an animal can lose practically all his glycogen and fat, and half his body protein, approximately 40 per cent of body weight, and still live, whereas the loss of 10 per cent of the water content of the body results in serious disorders, and the loss of from 20 to 22 per cent results in death.

The necessity of water in biologic processes is universal. Animals whose habitat is far removed from sources of water are favored with special mechanisms for their protection during water deprivation. Thus the camel not only has a system of stomachs most capacious and effective for carrying its water supply, but it has humps with great stores of fat which furnish large amounts of water of metabolism, a thick coat of fine hair which minimizes water evaporation, and a digestive tract that wastes no fluid, and permits dry stools only. Certain species of the moth,<sup>1</sup> are provided with a most effective mechanism whereby their

<sup>1</sup> The clothes moth, the bee moth, the four spotted pea weevil, and the Mediterranean flour moth.

own supply of water is furnished through metabolism. It has been demonstrated that they may live for long periods in a desiccator on food which contains but from 5 to 10 per cent of actual water and bring forth larvae containing from 50 to 80 per cent of water (Babcock).

In relation to the duration of life, water occupies a position intermediate between food and oxygen; is more vital than the former, and less vital than the latter. Some data bearing on the relative importance of these three essentials is shown in table 1.

THE CONSTITUTION OF WATER.<sup>2</sup> As commonly accepted, the constitution of water is  $H_2O$  and the molecular weight 18. That this holds true only under limited and unusual conditions has been proved recently largely through the work of Röntgen, Armstrong, Eötvös, Ramsey and Shields, and Sutherland. The percentage composition of water, two atoms of hydrogen to one of oxygen, was proved by Cavendish<sup>3</sup> in 1781, although the true explanation of his results was not apparent until the experiments of Lavoisier in 1783 (Bayliss).

The physical properties of water especially its freezing and boiling points, do not coincide with those of a simple compound containing three atoms only of gases with extremely low freezing and boiling points. If it were so constituted, its freezing point would be  $-150^{\circ}C.$  and its boiling point  $-100^{\circ}C.$ , in comparison with other similar compounds (Bayliss). Its high critical temperature, cohesion, refractive index, and high surface tension all indicate that its formula is more complex than  $H_2O$ . That this is true is widely accepted, at least for temperatures lower than  $400^{\circ}C.$

Practically all investigators are agreed that water is a multiple or polymer of  $H_2O$ . Röntgen first suggested that all abnormal properties of water could be accounted for qualitatively by assuming liquid water to be a saturated solution of ice in some other form of water. From a study of surface tension energy, Eötvös concluded that liquid water must be a polymer of  $H_2O$ . Ramsey and Shields computed from the surface tension that the formula at the boiling point must be  $(H_2O)_3$ , and in ice  $(H_2O)_4$ , a result arrived at earlier by Eötvös. Determinations of the freezing point of solutions of water in other solvents point to the formula  $(H_2O)_2$ . Sutherland has attempted to prove that steam is represented by  $H_2O$ , termed hydrol, and ice by  $(H_2O)_3$ , trihydrol, liquid water being  $(H_2O)_2$ , a mixture of trihydrol and dihydrol.

<sup>2</sup> For the discussion of constitution of water see: Millard: *Physical chemistry for colleges*. New York, McGraw, Hill, 1921, p. 63.

<sup>3</sup> The discoveries of Watt and Cavendish have been discussed at some length by Buckle, *History of Civilization in England*, New York, Appleton, ii, Pt. 2, p. 414.

TABLE 1  
*Rôle and behavior of oxygen, water, and food*

	PHYSICAL STATE	PERIOD BEFORE UTILIZATION	COURSE TO BODY CELLS	RESERVE STORE IN BODY	CONTROL OF INTAKE	PERIOD OF VOLUNTARY ABSTINENCE	DEATH FOLLOWS ENTIRE ABSTINENCE AFTER
Oxygen	Gas	Short—seconds or minutes	Lungs to blood, to cells	Small	Largely automatic	Seconds to minutes	Minutes
Water	Liquid	Somewhat longer, some minutes	Gastro-intestinal tract to liver, to blood, to cells	Considerable	Regulation of water balance. Thirst indicating need	Hours to 18 days	1½ days to 18 days
Food	Liquid or solid	Minutes to hours	Gastro-intestinal tract to liver to blood, to cells. Complicated metabolism	Great	Complex. Varies with types of food. Hunger indicates need	Days to 2 months (50 days)	104 to 117 days for dogs without death

As to the actual number of molecules existing in the various polymers opinions still differ. Bayliss says, "The balance of evidence appears to be that ice is trihydrol, steam monohydrol, and liquid water mostly dihydrol with varying amounts of the two other polymers according to temperature," and he looks upon water as a ternary mixture. From the foregoing it is obvious that water represents polymerized forms of  $H_2O$  but that the exact formulae of water in its various phases, ice, water, and steam, have not as yet been definitely determined.

The cause of the marked association of water is ascribed to the extra valence of oxygen. That oxygen is tetravalent at times is accepted by some authorities as proved.

When water combines with salts, the water may be present as hydrol. The degree of complexity of liquid water is supposed, according to Sutherland, to be altered not only by temperature variation but also when it acts as a solvent, a positive ion converting trihydrol into dihydrol and a negative ion causing a reversal of this change (Turner).

**WATER INGESTION AND EXCRETION.** Normally, water sufficient for the needs is taken daily in the form of liquids and food. Little or no water is absorbed in the stomach. It passes on into the intestine almost immediately, in small spurts occasioned by the contractions of the stomach. Taken without food, the passage of water into the intestine is but a matter of a few minutes or at most of from  $\frac{1}{2}$  to 1 hour; 495 cc. of 500 cc. ingested has been recovered from the duodenum within 25 minutes (Von Mering). When taken with food, 2 or 3 hours or more are required. Constriction of the intestinal vessels retards and dilatation increases absorption (Sollmann, Hanzlik and Pileher). From the intestine it is absorbed rather quickly, the rate depending in part on whether or not it is mixed with food and also probably on the degree of saturation of the body. A small proportion probably remains in the intestine and passes from the body in the stools. It is absorbed from both small and large intestine. After absorption it becomes a part of the water of the organism and plays its rôle in one or another of the various phases of metabolism or in the execution of some physiologic function on the part of some tissue or organ. It may circulate in the blood or lymph stream, it may play a part in any of the numerous hydrolytic or oxidative reactions; it may constitute the water content of the tissues or of one organ as a liquid phase of colloidal solution or as water of hydration, it may serve as the vehicle of transportation; it may be excreted by one organ, only to be carried elsewhere to another field of chemical reaction, or it may act as a lubricant on some glistening surface subject to friction.

It reaches every cell in the organism, and through its properties furnishes the opportunity for chemical reactions, for changes in physical state, and for energy transformation.

Having served these functions it continues to serve in its excretion by way of the kidneys, bowels, lungs or skin, and appears finally in the urine, feces, sweat, visible or invisible, or as moisture in expired air. The proportions excreted by these different channels vary greatly from time to time in the same individual even in health, depending upon the influence of almost innumerable factors, many of which will be considered later.

In health, despite the complexity of the rôle in metabolism, water is ingested and excreted in balanced amounts so that the water content of the tissues remains practically constant and at a level of maximum efficiency. The mechanism works smoothly and automatically. The sensation of thirst indicates the needs of the body, and the sensation of distention of the bladder or bowels the desirability of evacuation.

*The maintenance of supply.* If water is excreted it must be replaced in order that metabolism may proceed at its maximum efficiency. The need of the body for water is determined largely by environment and metabolism. Water is ingested intermittently as drink or food and ejected intermittently in the urine and stools. But it is supplied continuously to the cells and is also lost continuously by way of the lungs and skin.

*Clinical studies of water balance.* As ordinarily conducted, clinical studies of water balance are grossly inaccurate, in that they take account as a rule only of the fluids ingested and of the fluids excreted in the urine and feces. Fortunately the weight of the patient is usually followed, which serves as a valuable control.

The accurate determination of water balance is a matter of considerable complexity and cannot be carried out satisfactorily except in a well equipped metabolic laboratory. The table of Soderstrom and DuBois illustrates the various factors entering into consideration of water balance and indicates the need of scientific training and of technical facilities in undertaking its determination (table 2).

*A historical sketch of water balance studies.* The first reference to the importance of determining the water balance is made by Celsus in *De Medicinæ*, in which he says, in a discussion of dropsy, "Nor is it improper to measure both the drink and the urine; for if more fluid is excreted than is taken, so at length there is hope of good health." He further quotes Asclepiades as having instituted abstinence for two days

in dropsy secondary to malaria. Araeteus, the Cappadocian, noted disturbances in the urinary output in diabetes. He says of it: "Diabetes is a wonderful affection, not very frequent among men, being a melting down of the flesh into urine." However, he left no records of quantitative studies of water exchange. Sanctorius, a friend of Galileo, was the first to attempt comprehensive studies in water metabolism. While the latter was engaged in the creation of the science of physics and mathematics, Sanctorius, through his assistance, was engaged in applying the thermometer and balance, that is, the tools of science to problems of physiology. In 1614, in his book, *De Statica Medicina Aphorismorum*, he published the results of years of investigation on

TABLE 2  
*Water balance*

Water intake:	grams	
Drinking water.....	300	
In coffee, milk, and soup.....	580	
In solid foods.....	720	
From oxidation of 100 grams of protein.....	41	
From oxidation of 100 grams of fat.....	118	
From oxidation of 244 grams of carbohydrate.....	135	
	<hr/>	1894
Water output:		
In urine.....	750	
In feces.....	300	
Vaporized through skin and respiratory tract.....	700	
	<hr/>	1750
Plus balance to body.....		144
Gain in body weight.....		100

increases and decreases in his own body weight and upon the factors determining these variations. He compared the weights of food and drink taken daily with the weight of the urine and feces discharged. From these data and the variations in the weight of his body he deduced the "quantity of material lost by insensible transpiration through the skin and lung."

Only when science had advanced to the stage where the nature of the chemical processes concerned could be appreciated (1840-1850) were more comprehensive studies of metabolism undertaken. Then in rapid succession came many fundamental investigations dealing with general metabolism including that of water, which were followed some time

later by the important studies of water loss of Pettenkofer and Voit, and Rubner, and Wolpert. Within very recent years have come the more exact calorimetric studies of Atwater and Benedict, of Benedict and Carpenter, and of DuBois, and the ingenious study of Lombard of the weight loss each minute by transpiration.

**THE WATER INTAKE:** *Water and beverages.* Generally speaking more water is ingested than is required by the economy. The chief source of the fluids of the body is water per se, large quantities being consumed also in the form of beverages, such as tea, coffee, cocoa, milk, carbonated waters, and spiritous liquors. Their ingestion results in part from appetite and habit rather than from true thirst. The rôle of age and several other factors will be discussed later.

*Water and solid food.* More water is ingested in food than is generally recognized. In an ordinary diet as much as a liter of water a day may be taken in the form of so-called solid foods, taking into account the water contents of these foods and also the water resulting from their oxidation. In table 3 are tabulated a number of so-called solid foods, their percentage of water, weight of average servings, and their caloric values. From this table has been purposely omitted all food such as soups, custards, and compotes in which the high content of water is generally recognized. The number of foods with water content of more than 50 per cent is certainly striking.

The distribution of food in nature is singularly interesting. In the tropics, where the loss of fluid from the body is excessive, foods rich in water abound. In the arctic regions, fat constitutes a large part of the diet and water of oxidation plays a relatively larger part.

*Water of metabolism.* The sources of water of metabolism are *a*, oxidation of organic matter in intracellular respiration; and *b*, other changes in the molecular structure of substances entering into the composition of cells and tissues, such as syntheses, concerned in the building up of dextrose into cellulose or starch, or of amino acids into complex proteins.

Magnus-Levy calculated the amount of water formed in the oxidation of foodstuffs as follows:

100 grams of fat	give 107.1 grams of water
100 grams of starch	give 55.5 grams of water
100 grams of protein	give 41.3 grams of water
100 grams of alcohol	give 117.4 grams of water

An ordinary mixed diet yields in the neighborhood of 300 grams of water. Magnus-Levy stated that a 2000 calorie diet yields approxi-

TABLE 3\*  
*Water content above 50 per cent*

FOOD	WATER	AVERAGE SERVING	CALORIES	FOOD	WATER	AVERAGE SERVING	CALORIES
	<i>per cent</i>	<i>grams</i>			<i>per cent</i>	<i>grams</i>	
Cucumbers.....	95.4	50	8.92	Cod steaks, fresh....	79.7	100	81.32
Lettuce.....	94.7	25	4.9	Smelts.....	79.2	100	88.9
Wax beans, canned...	94.6	100	17.74	Lobster.....	79.2	100	83.98
Celery.....	94.5	50	9.48	Lima beans, canned..	78.5	100	79.0
Asparagus, canned...	94.4	100	18.56	Macaroni, cooked...	78.4	100	91.03
Tomatoes, fresh.....	94.3	100	23.4	Brook trout.....	77.8	100	98.25
Tomatoes, canned....	94.0	100	23.18	Green corn, canned..	76.1	100	100.52
Brussels sprouts, canned .....	93.7	100	21.02	Potatoes, boiled....	75.5	100	88.67
Beef juice... ..	93.0	100	25.67	Halibut.....	75.4	100	124.62
Whey.....	93.0	100	27.39	Bananas.....	75.3	100	101.11
Water melon.....	92.0	100	29.81	Plums.....	74.5	100	82.0
Radishes.....	91.8	50	15.02	Cream, 18 per cent..	74.0	100	200.75
Onions, boiled.....	91.2	100	41.75	Mackerel.....	73.4	100	142.7
Buttermilk.....	91.0	100	36.63	Egg, boiled.....	73.2	50	56.3
Strawberries.....	90.4	100	40.0	Rice, boiled.....	72.5	100	112.45
Spinach, cooked.....	89.8	100	22.06	Cottage cheese.....	72.0	50	56.3
Beet greens, cooked..	89.5	100	25.80	Veal leg.....	71.7	100	147.24
Peaches.....	89.4	100	42.34	Liver....	71.0	100	132.46
Pineapple.....	89.3	100	44.2	Sweet breads.....	70.9	100	191.41
Koumiss.....	89.3	100	53.15	Tongue.....	70.8	100	163.5
Beets, cooked.....	88.6	100	40.5	Chicken, white meat	70.3	100	158.57
Oysters, solids... ..	88.3	100	50.2	Lamb leg, roast... ..	67.1	100	198.81
Squash, canned.....	87.6	100	51.39	Pork tenderloin....	66.5	100	198.39
Milk.....	87.0	100	71.23	Salmon, fresh.....	64.6	100	209.24
Oranges.....	86.9	100	52.7	Salmon, canned.....	63.5	100	208.47
Egg whites.....	86.2	32	17.6	Sirloin steak.....	61.9	100	249.54
Peas, canned.....	85.3	100	56.8	Bologna.....	60.0	100	240.35
Apple.....	84.6	100	64.57	Turkey, white meat.	58.5	100	187.43
Boiled oatmeal.....	84.5	100	63.28	Pork and beef sau- sage... ..	55.4	100	303.67
Pears.....	84.4	100	64.9	Sardines, canned....	52.3	100	277.51
Haddock, fresh. ....	81.0	100	73.3	Corned beef.....	51.8	100	276.16
Clams, solids... ..	80.8	100	75.0	Ham, boiled, smoked	51.3	100	290.84
Prunes.....	79.6	100	80.18	Mutton leg, roast...	50.9	100	312.68

\* Table prepared by Mary Foley, dietitian of the Mayo Clinic.

mately 240 grams and a 4000 calorie diet, 480 grams of water, or about 12 grams for each 100 calories.

Intracellular metabolic water results in dilution of cell contents, which disturbs the osmotic equilibrium between the fluids within and without the cell, which occasions movement of nutriments by osmosis to these centers of dilution and of water and carbon dioxid in the opposite direction.<sup>4</sup> As a result the water of metabolism plays a unique part and one that cannot be duplicated by water derived from outside the cell. This holds true for protoplasm of both the animal and the vegetable kingdom. Relatively, the water of metabolism forms a large percentage of the water required for vital processes in animals; indeed in some animals it supplies the entire need of the economy over long periods of time. In animals, also, the water demand for the excretion of waste products is large, but in those having little or limited access to water the end products of nitrogen metabolism appear in solid form, that is uric acid, which is relatively non-toxic and the hydrogen content of which is very low. In many of these animals the metabolism is such as to protect them to the uttermost against loss of water. This is especially striking in certain animals in arid districts, serpents, prairie dogs, mice, sheep, birds, and, as already mentioned, camels, and certain varieties of moths (Babcock).

*Hibernation.* In hibernation animals depend for long periods solely on their water of metabolism. Well nourished and well padded with fat, they enter their long sleep. Metabolism proceeds at an extremely low level, but moisture is constantly given off by the lungs. The fat furnishes the greater part of the water, protein metabolism being at a minimum. In some instances the thick coat of fur, close quarters, and low temperature usually keep water loss by way of the skin at the lowest possible level (Babcock).

*The nature of the sensation of thirst.* Thirst is the index to the body need of water. It is a sensation of dryness of the mouth and throat accompanied by a desire for water. It develops automatically with the need of furnishing the fresh supply of water to the organism. Like hunger it is a part of the replenishing mechanism of the body, and like hunger it has been the subject of considerable physiologic investigation. Hunger and thirst must be distinguished from appetite, which develops as the result of habits in eating and drinking.

<sup>4</sup> This view which has been presented among others, for instance by Babcock, presupposes the presence of cell membranes.

No doubt exists concerning *a*, the reference of thirst sensation to the mucous membrane of the mouth, pharynx, root of the tongue, and palate; or *b*, the scantiness and stickiness of saliva and mucous in the mouth during thirst. The latter has been emphasized strikingly by King, a medical officer who studied a troop of cavalry which was lost for  $3\frac{1}{2}$  days without water in the torrid "Llano Estacado" in Texas. He states that long before the third day salivary mucous secretions had disappeared from the mouth and that "brown sugar would not dissolve in the mouth."

Dryness of the mouth may result from local or general causes. Among the local causes are breathing hot dry air, prolonged speaking and prolonged chewing of dry food. In public speaking or singing, fear may constitute an additional factor.

The general causes include factors reducing the general fluid content of the body through excessive loss resulting from profuse sweating, diarrhea, polyuria, lactation or from hemorrhage and shock. Two rival theories of thirst have arisen; one ascribes it to a local sensation and the other to a general sensation or tissue thirst.

The chief argument favoring local sensation is the abolition of thirst by the application of local anesthetics to the mucous membrane of the mouth and throat in diabetes insipidus (Lepidi-Chioti and Fubini) and in dogs (Valenti). Against this, Wassilief has argued that swallowing is made difficult or impossible by local anesthesia, which in turn is denied by Valenti. In arguing against it we might also refer to our own experience in which the local application of cocaine, pushed in one case to the point of constitutional toxicity, failed to decrease the water intake in diabetes insipidus. In its favor we might suggest that the sensation of hunger has been proved to be local in origin and that the sensations attending bowel and bladder distention are also local in character.

The view that thirst is a general sensation is stated by Schiff: "It arises from a lessened water content of the body, a condition from which the whole body suffers. The local reference to the pharynx, like the local reference of hunger to the stomach, is due to association of experiences. Thus the feeling of dryness in the mouth, although it accompanies thirst, has only the value of a secondary phenomenon, and bears no deeper relation to the general sensation than the heaviness of the eyes bears to the general sensation of sleepiness."

The evidence on which the theory of general sensation is based consists of *a*, the abolition of thirst in dogs through the injection of water into the veins (Dupuytren, Orfila); and in a patient suffering from

hydrophobia (Magendie); *b*, the striking thirst and the failure of ingested water to relieve it in dogs with open gastric fistulas, an experiment devised by Claude Bernard. He says, "As the animal became thirsty, it would drink until fatigued, and when rested it would begin again; but after the fistula was closed, drinking quickly assuaged the desire for water;" *c*, dogs continue to drink water after the section of the glossopharyngeal, lingual, and vagus nerves on both sides (Longet); and *d*, increase in osmotic pressure occurs in thirst as indicated by the freezing point in conditions naturally accompanied by thirst (Mayer).

According to Cannon these findings do not necessarily indicate that the sensation of thirst is general. Each is capable of interpretation other than that suggested by the investigator, thus: *a*, the abolition of thirst by parenteral administration of water may result in more copious secretion of saliva which, acting locally, relieves thirst; *b*, Bernard's interpretation has been challenged by Voit, who points out that the drinking of water under these conditions moistens the throat only temporarily and does not compensate for decreased salivary secretion resulting from depletion of the body of water; *c*, the continuous drinking after section of the nerves to the mouth may be due to habit or appetite, even if all the branches of the nerves are severed, which is usually not the case (Voit); and *d*, increase in osmotic pressure in thirst does not arise until after two or three days of water deprivation, at which time animals with slightly hypertonic blood will drink salt solutions with apparent relief, while dogs with markedly hypertonic blood drink hypertonic salt solution repeatedly without relief (Wettendorff).

Cannon believes that "in each condition a general bodily need has arisen from a lack of essential bodily material and is signaled by a well-defined thirst. In each the testimony of ingenious persons regarding their feelings has been carefully set down and then explained away." He calls attention to the fact that animals living on land have special buccal glands, while those living in water do not, and cites the work of Bidder and Schmidt in which, following the ligation of all the salivary ducts, the animals were always eager for water. He further relates personal experiences: *a*, mouth breathing resulting in sensation of dryness; *b*, the influence of prolonged water restriction decreasing the salivary output and its immediate increase following water ingestion; and *c*, the decrease in salivary secretion following the loss of 500 cc. of body fluid as sweat. In each instance the relation between the decrease of salivary flow and the sensation of thirst was quite clear.

Thirst, according to Cannon, is a local sensation resulting from local dryness of buccal and mucous membrane, due to a decrease of the secretions from the salivary glands which in turn is dependent on diminished supply of fluid furnished these glands by the body because of its depletion in water. Lack of water secreted by the salivary glands is the cause of the local sensation of thirst, and thirst indicates the need of replenishment of fluid in the body.

RÔLE OF WATER IN THE ORGANISM. *Intermediate water exchange.* Only one phase of this subject is presented by way of illustration. Much fluid after absorption finds its way back into the alimentary tract. In fact, the quantity entering the intestines as digestive fluids, far exceeds that taken by mouth.

The amount of these secretions is large. An approximation of the daily excretions in a man of average size is shown in table 4.

TABLE 4

SECRETIONS	AMOUNT	SPECIFIC GRAVITY	AUTHORITY
	cc.		
Saliva.....	1500	1.002	Bidder and Schmidt
Gastric juice.....	2000 to 3000	1.006 to 1.014	Bidder and Schmidt
Bile.....	300 to 500	1.026 (?)	Pfaff and Balch
Pancreatic juice....	500 to 800	1.0075	Wohlgemuth
Succus entericus....	3000		Pregl

From this it appears that the amount of fluid excreted into the intestine daily is from 7,500 to 10,000 cc. or from two to three times the amount of fluid ordinarily ingested by mouth and from three to four times the amount ordinarily excreted as urine or about twice as much as the total volume of the blood.

As the contents of the intestine pass downward, reabsorption of fluid takes place, the amount remaining in the feces rarely exceeding 200 cc. each day.

Although considerable is known concerning the influence of certain factors on the amount and composition of these various digestive fluids, the subject will not be discussed here.<sup>5</sup>

*Water and heat regulation.* Water regulates the temperature of the environment and the organism. This is made possible through its unique thermal properties. The specific heat of water is the highest

<sup>5</sup> According to Mathews, the duodenum excretes a large quantity of an alkaline albuminous juice, 50 cc. being collected in one experiment within 2 hours in a dog weighing 5 kgm.

known for any substance, solid or liquid, with one exception. The latent heat of vaporization of water is the highest known, while the latent heat of cooling, is next only to that of ammonia. The latent heat of vaporization is of universal significance in relation to dissipation of body heat because evaporation occurs at all temperatures. The amount of vapor that air can hold when in contact with liquid is variable, is dependent on temperature and pressure, and is greatest for fluids whose latent heat of vaporization is greatest. Consequently more water can vaporize than any other substance. Heat conduction of water, although low as compared with that of some metals, is yet the greatest known for any liquid. Hence water serves best of all liquids in heat conduction in and away from the body. It is the most ideal buffer for heat in existence, that is, it exerts unparalleled resistance to heat or cold before changing its temperature or its physical state, while in addition its high latent heat of vaporization is of constant value to the organism in the dissipation of heat.

Water, by virtue of mobility is readily shifted so as to meet the constantly changing needs in relation to heat regulation in the body. By virtue of its stability, it places its thermal properties at the disposal of the organism for this important function. Besides, of all liquids, it is the most available.

Water, then, regulates heat distribution and dissipation through its mobility and its ideal thermal properties: *a*, high specific heat, which favors storage; *b*, high caloric demands for its evaporation, which permit a rapid elimination of heat; and *c*, high heat conductivity which provides rapid equalization of heat within the tissues of the body (Barbour). The regulating mechanisms in regard to its dissipation are: 1, the vasoconstrictor center and the vasoconstrictor fibers in the skin; 2, sweat and the sweat centers and nerves; and 3, the respiratory center. The nervous mechanism is responsible for the mammal being homeothermic rather than poikilothermic, and destruction of this mechanism results in its conversion from the former into the latter state.

*Physico-chemical properties of water.* No less unusual and important than the thermal properties of water are those dealing with physical and chemical processes which take place when other substances are brought into contact with it; processes involving solvent power, ionization, hydration, imbibition, and surface tension. By virtue of its dissociating power water may acquire catalytic activity. It is very questionable whether water ever exists in the organism as such; it is present in the form of a complex salt solution and as such conducts electricity and does not act as an insulator as would free water.

*Solvent properties.* In the organism water is the universal solvent, but even the process of solution of one salt in water is not entirely clear. However, it is evident that dissociation occurs and that in solution the molecules of a substance are free to manifest the effect of energy due to their movements. The process of solution is one of dispersion, similar in kind to that occurring in colloid solutions but differing as to the degree of subdivision and fineness of the dispersoids.

When one considers the number and character of electrolytes, non-electrolytes, colloids, and gases occurring in the blood and urine, the solvent action of water becomes apparent. Although all these substances are freely soluble in water, the majority of them are not soluble in other liquids, not even in alcohol.

*The water content of the tissues.* Speaking generally, the amount of water in the body is fairly constant. It varies somewhat with age. The fetus contains a great deal of water; the younger the fetus, the more the water (Fehling). At birth the amount of water in the organism is relatively small to judge from the water content of the blood. It increases at about six months at which time aging begins (Lederer). In old age the tissues are tough and appear to be dry, but according to Ranke, the water content is increased to 81.2 to 84.8 per cent instead of 75 to 80 per cent.

Fat alters the percentage content of water in the body, "generally about twenty to twenty-two parts of protein are soaked in and swollen with seventy-eight to eighty parts (four times as much) water. Fat enters into the interstices of the protoplasm as a dry and waterless mass, neither driving water out from the tissues nor bringing it in to them" (Magnus-Levy).

Engels has studied the water depots of the body following intravenous injection of salt solution. He found that all the tissues except bone became more watery. The muscles, skin, and kidneys take up the greater amount of water, increasing their percentage content of water 3.86, 3.23, and 3.83, respectively. The muscles representing 40 per cent of the body weight take up more than two-thirds of any added water, but continue to act normally in spite of the added moisture.

*The state of water in the organism.* Water exists in the organism mainly as a salt solution, as the liquid phase of colloidal solution, and as water of hydration. It is of vital importance since its properties and those of the cell determine the amount of the various substances present and their exchange. The turgor of cells is dependent on their water content which in turn is dependent on osmotic pressure or the hydration capacity of the protoplasm itself.

The crystalloids in the saline solution of the body exist in rather fixed concentration as is represented in Ringer's solution: sodium chlorid 0.7 per cent, potassium chlorid 0.03 per cent, and calcium chlorid 0.025 per cent. Locke's solution contains in addition sodium bicarbonate 0.01 to 0.03 per cent and glucose 0.1 per cent. Locke's solution approaches in composition deproteinized plasma and contains both electrolytes and nonelectrolytes.

Blood plasma is composed of water and salts, as in Locke's solution, and in addition proteins, as serum albumin, serum globulin, and fibrinogen, gases, metabolites, enzymes, and special substances such as hormones, and so forth. Of the proteins the albumin forms somewhat more than 50 per cent, globulin somewhat less than 50 per cent, and fibrinogen from 5 to 10 per cent.

*Water and protoplasm.* For want of space, water and protoplasm and water as a medium of transportation will not be discussed.

*Water as a lubricant.* In most mechanical devices involving motion, lubrication is necessary. Numerous surfaces in the organism are subject to more or less constant friction, which would result in irritation were it not for lubrication. Thus moisture prevents the serious consequences of friction in the pleurae, peritoneum, joints, and eyes. In the body all surfaces subject to friction automatically control their own lubrication through the excretion of fluid on the surface involved. In most instances water plays the major part.

**WATER OUTPUT.** *Factors determining the relative loss of water by various channels.* The total output of water is determined by the total intake. The relative output by the various channels differs widely in different individuals and in the same individual under varying conditions. An illustrative water balance from DuBois has already been presented. Careful studies have also been made by Atwater and Benedict, and others.

The human organism as compared to animals varies greatly in its responses to stimuli, partially because of differences in reactivity. It is a matter of common experience to find one patient with sweat literally dripping down the sides, while the skin of another patient, examined under identical environmental conditions, remains entirely free from moisture. With some persons public speaking is accompanied by marked dryness of the respiratory passages, while with others no difficulty of this kind is encountered. Polyuria results from nervous tension in some individuals, while others under the same external conditions, do not experience it. Thus many imponderable influences

play a part in water exchange and in water retention in the body. "And some, when the bag pipes sing in the nose, can scarce contain their urine."

Since so many factors play a part in determining the elimination of water by each channel, no hard and fast rules can be laid down as to the relative amount of water excreted by each. Some of the more important factors affecting these losses will be discussed separately in some detail.

Attention might be called to the fact that the amount of water lost by the feces is the smallest and ordinarily subject to least change, while the amounts lost by the kidneys and skin are greatest and subject to the greatest variations. Roughly speaking, the amount of fluid lost by these channels tends to vary inversely, other things being equal.

Heat is lost to the organism through *a*, urine, feces and saliva, which are expelled at body temperature; *b*, expired air (air enters the body

TABLE 5

CHANNELS	PER CENT	CALORIES
Urine and feces.....	1.8	48.0
Expired air in warming.....	3.5	8.4
Vaporization of water from lungs.....	7.2	18.2
Evaporation from skin.....	14.2	36.4
Radiation and conduction from skin.....	73.0	1792.0

at environmental temperature and humidity and leaves it, saturated and at a higher temperature); *c*, evaporation from the skin of sweat and invisible perspiration; and *d*, radiation and conduction from the skin. Under certain conditions heat loss occurs almost entirely (98 per cent) by way of the skin as may be seen in table 5.

In the calorimeter 24 per cent of the total heat production ordinarily is lost by vaporation. As environmental temperature increases and conduction and radiation become insufficient, the evaporation of water is of ever increasing importance. At 37°C. heat loss is effected entirely through vaporization. The factors usually affecting heat dissipation are: climatic conditions; environmental temperature, relative humidity and winds; rate of metabolism and clothing. Heat loss is a surface phenomenon and the amount of heat reaching the surface is the fundamental factor. Heat is brought to the surface by blood, that is, water. The cutaneous factors are: the emissive powers of the surface; the evaporation of the surface; and velocity of trans-

portation of heat to the surface depending on the conductivity of tissues and the speed of cutaneous circulation (Hill). Here, we are concerned with heat dissipation only in so far as loss of water enters into consideration.

*Water elimination by skin and respiratory tract.* Most of the determinations of water loss through the skin and respiratory tract have been made under what might be considered standard conditions, that is, fasting at average mean temperature and humidity with approximately the same amount of clothing. As early as 1866, Pettenkofer and Voit showed that daily water elimination by these channels in a fasting man reached as high as 829 gm. This has been abundantly corroborated by the work of Rubner, Schwenkenbecher, Wolpert, Sudovyen, Benedict and Carpenter, and DuBois. Wolpert found that the hourly production of water vapor calculated to a uniform body weight of 70 kgm. varied from 30.9 to 70.9 grams; the seven subjects vaporized from 56 to 60

TABLE 6

	WEIGHT	WATER ELIMINATED EACH DAY	CALORIES LOST IN EVAPORATION
	<i>gm.</i>	<i>kg.</i>	<i>per cent</i>
Man.....	70,000	12.6	22.0
Dog.....	30,000	12.2	20.0
	4,000	11.5	9.0
Guinea pig.....	550	11.3	6.5

grams of water each hour at a temperature of 18°C. with a relative humidity of from 35 to 60 per cent. Under extreme conditions the hourly loss may be increased to as much as 1 kgm.

Rubner, utilizing a Pettenkofer-Voit chamber, discovered that water elimination is proportional to body weight and not to body surface, that variations in humidity do not affect heat production, and that an increase in moisture in the air decreases the heat lost by evaporation but correspondingly increases that lost through radiation and conduction.

The relation of body weight to water elimination is illustrated in Rubner's table. (Table 6.)

The most comprehensive study of the question has been made by Benedict and Carpenter utilizing an Atwater-Rosa calorimeter. They found that "the average for 158 days, covering 2150 hours, shows that the insensible perspiration of several healthy men, sitting, lying asleep or awake, or engaged in minor activities, is approximately 40 grams an

hour." Soderstrom and DuBois utilizing the Sage calorimeter found that normal men between the ages of 20 and 50 years excrete on an average of 29 grams of water an hour, about 700 grams each day, and state that few men depart more than one-tenth from this amount under standard conditions. As a rule the proportion of water lost by the lungs is smaller than that by the skin.

*Methods of determining the elimination.* The amount of water lost by way of the skin and respiratory tract is difficult to determine accurately. It can be estimated approximately by taking into consideration the daily weight of the body and the amount of material ingested and excreted, as was done in the beginning of the seventeenth century by Sanctorius and in the twentieth by Lombard. However, the best results are obtained through the use of the direct calorimeter constructed with a view of determining water lost by vaporization. Through establishing an air current and determining the amount of air circulating in the system and its water content on entering and leaving, the water of vaporization can be determined.

But even with such facilities the error in determining the loss of fluid by evaporation is large, at times, and absolute accuracy is rarely attained. For the Sage calorimeter, which is especially well adapted for work of this kind, the error is probably plus or minus 3 per cent. Control experiments such as those carried out by DuBois, in which alcohol checks were employed along with the dripping of known amounts of water, showed the average error in determining the water of vaporization to be as high as 6.7 per cent. DuBois says it is doubtful if the determination is much more than 10 per cent accurate under ordinary conditions. For determining the water lost by the respiratory tract a hood was devised by Zuntz; and for that lost by skin, cabinets and chambers enclosing the entire body to the chin are utilized (Rubner and Nuttall). In the study of the loss of water from localized areas, small air chambers have been attached locally to the surface of the skin.

*Factors affecting water lost by lungs and skin (climatic conditions).* Climatic conditions are of paramount importance and involve factors of temperature, relative humidity, vapor tension, air currents, radiant heat, seasons, day and night, and clothing. These factors may act independently or collectively, in unison or opposition, proportionately or disproportionately. Such a combination precludes prognostication in any given case and necessitates actual determination of water loss. Separate consideration of the nature of the influence will be given each of these factors.

*Temperature.* Other things being equal, the higher the environmental temperature, the greater the loss of water by evaporation. The capacity of air for holding water vapor increases with the temperature.<sup>6</sup>

Temperature of air, °C.	-1	4.5	10.0	16.0	21.0	26.5	32.5	38.0
Maximum weight of water absorbed by the air, grains for each cubic foot.....	1.94	2.85	4.08	5.75	7.98	10.9	14.7	19.7

Rubner studied the water vaporized by animals and found that water elimination in guinea pigs increased as the temperature of the air was raised above 15°C. and in dogs it increased with temperatures above 7°C. As the temperature increases, increased rate of respiration develops. In dogs panting is especially marked in hot weather. Under ordinary conditions in man sweating begins in the neighborhood of a temperature of 37°C. Sweating may cease in extreme heat and heat stroke is then imminent.

*Temperature and humidity.* Rubner made a careful investigation of water loss in both man and animals. He found that animals give off more water in dry than in moist air, an increase of from 200 to 300 per cent, accompanying a decrease of relative humidity from 69 to 31 per cent. In a man in the chamber, Rubner found the water given off from lungs and skin to be:

TEMPERATURE	WATER ABSORBED BY DRY AIR		WATER ABSORBED BY MOIST AIR	
	Lungs	Skin	Lungs	Skin
°C.	gm.	gm.	gm.	gm.
15	16.8	9.5	9.0	
20	17.0	37.1	11.7	3.6
25	18.4	57.0	10.9	13.0

Benedict and Carpenter also studied the effect of relative humidity. They found that at a humidity of 30 per cent, 60 grams of insensible perspiration were lost, at 50 per cent, 26 grams, and in one individual studied over a period of 27 days, there were variations from 23 to 35 grams.

Lyon has determined that the average evaporation from lungs and skin of a large laboratory class, when the temperature was below zero

<sup>6</sup> One cubic meter of air saturated with water vapor at 37°C. contains 43.465 grams of water (Smithsonian table).

outside and 70°F. inside, was in the neighborhood of 60 grams an hour for each person, or about 1364 grams a day.

*Air currents.* The movement of air is also a potent factor in evaporation of water from the body. Ordinarily it plays an important part under conditions of the natural outside environment. Wolpert found that when the air temperature was between 15 and 35°C., the wind from a fan diminished the water loss from the body; above and below

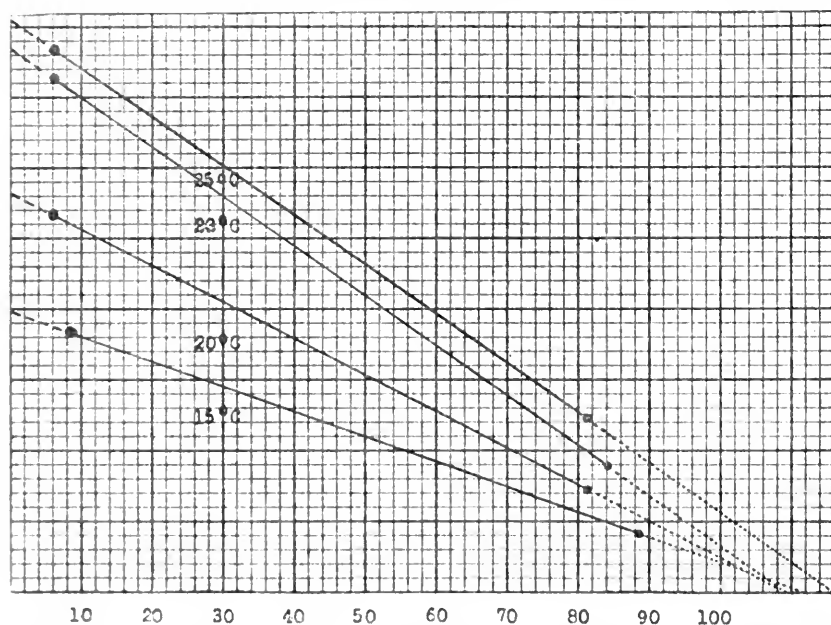


Fig. 1. After Rubner and Lewschew. To show the relation of the loss of water in the form of vapor to temperature and to air moisture. The numerals along the base indicate the degree of air moisture. The verticals represent the water vapor loss in grams.

these temperatures, it increased the evaporation. Its influence is intimately connected with evaporation, temperature, and humidity.<sup>7</sup>

According to Hill, it appears that the relative humidities around 35 per cent are at least more comfortable than either the extreme dryness

<sup>7</sup> Since at each air temperature above blood heat the bodily gain of heat from the air by conduction increases as the strength of the wind increases, and since the human body cannot perspire above a certain maximum rate, there must, at least on theoretical grounds, be a certain corresponding critical value of the wind velocity which if exceeded must produce a net gain of heat to the body. The

or the 50 per cent humidity, which feels quite moist. The influence of temperature and vapor pressure on the amount of water vaporized from the skin is illustrated graphically in the chart taken from Rubner and Lewschew.

*Day and night.* Day and night are accompanied by marked changes in the atmosphere. The temperature changes are matters of common knowledge. Relative humidity is subject to variations just as striking. Rubner quotes some figures showing humidity changes by day and night in Vienna. Thus in winter the relative humidity at midday was 75 per cent, at midnight 95 per cent; in summer at midday 49 per cent, and at 6 a.m., 77 per cent. Season also has a marked effect, the average humidity for January being 84 per cent and for April and July 63 per cent.

*Effect of food and fasting.* The ingestion of food increases the water output especially at high temperature. Rubner found that when dogs were given meat, the water elimination was not affected when the environmental temperature was low but that water elimination was increased markedly when the air was warm. The specific dynamic action of food unquestionably plays a part.

Benedict and Carpenter have shown that a change from a diet poor in carbohydrate to one rich in carbohydrate is accompanied by an increase in weight and by considerable retention of water by the body tissues. Conversely, a change to a diet poor in carbohydrate but rich in fat is accompanied by loss of water from the body. With the caloric intake approximately the same on a diet rich in carbohydrate, there was an average gain of 88 grams of water a day, while in the same subject on a diet high in fat and low in carbohydrate, there was an average loss of 906 grams of water a day. This offers a fertile field for further investigation.

Benedict found that the greatest output of water vapor occurred during the waking hours but failed to find any increase as the result of the

less the temperature of the air exceeds body temperature, the higher will be the critical wind velocity (Hill).

It has been shown that at an air temperature of 45°C. and relative humidity of 40 per cent, a wind of 9 meters per second results in warming, while in a calm cooling occurs.

Huntington has discussed climate in relation to efficiency.

The kata thermometer will probably play a considerable rôle in future investigations of the factors.

Acclimatization or training is an important factor in withstanding the effects of the tropical sun (Shaklee).

ingestion of water. As a rule water lost by the lungs was somewhat less than that from the skin. Benedict and Carpenter have findings in investigations extending over long periods of fasting and have calculated the water vaporized from the lungs as amounting to 9.6 and 10.7 grams each hour and as 40 to 65 per cent of the total amount vaporized.

DAY OF FAST	RELATIVE HUMIDITY	TOTAL WATER VAPORIZED
	<i>per cent</i>	<i>grams</i>
1 to 3	52 to 63	23.29
17 to 20	39	15.16
27 to 30	42 to 49	18.20

Laschtschenko showed that there was no increased loss of water by the skin and lungs following the drinking of water up to 2 liters.

*Exercise.* Light exercise in cool weather has but a slight influence, but hard work in hot weather increases the loss tremendously. Benedict and Carpenter found that with average muscular activity there was a variation from 140 to 276 grams each hour, while at rest under the same environmental conditions the output was from 23 to 79 grams each hour. Obesity tends toward a greater loss of water during work (Schattenfroh).

Wolpert found that a man at rest in still air at 21°C., evaporated 42 grams of water an hour, giving a minimal evaporative heat loss of 576 calories a day. While working at the rate of 15,000 kpm. an hour, he evaporated, under the given conditions, as follows:

TEMPERATURE	RELATIVE HUMIDITY	WATER EVAPORATED EACH HOUR
<i>°C.</i>	<i>per cent</i>	<i>grams</i>
7	81.0	58.0
13	81.0	70.8
17	87.0	90.4
19	81.0	112.8
25	47.0	230.0

*Rest and sleep.* Benedict and Carpenter compared the output in rest and sleep. In rest the loss for each kilogram was 0.61 gram an hour with a variation of from 1 to 0.42 gram, while in sleep, the average loss was 0.48 gram with variations from 0.36 to 0.68 gram. The average value in rest for each square meter of body surface was 19.7 grams an hour,

with variations from 29.2 to 14 grams while in sleep the average was 15.9 with a variation of from 22.2 to 12.4. During rest the average output is 40 grams an hour, while that in sleep is 32.1 grams an hour.

They further determine the relative amount of water vapor lost from the lungs and skin, utilizing the method of computation devised by Zuntz. This assumes that the inspired air has the moisture content of the air of the room and that the expired air is saturated at the temperature of the body, namely 37°C. Thus by knowing the total ventilation of the lungs it is possible to compute the output of water vapor through the lungs. Zuntz and his associates have determined that for every litre of oxygen absorbed there is a ventilation in the lungs of 21 litres of air, and consequently multiplying the amount of oxygen absorbed by 21 yields the total ventilation of the lungs in liters of air. Although the method is fallacious in some respects, especially during exercise, Benedict and Carpenter believe that it yields fairly accurate and rather constant results during rest. Their results show that in the same individual at rest the ratio of the amount of water vaporized by the lungs and skin is fairly constant and that on an average the amount lost from the skin is approximately 56 per cent of the total, and that this ratio is not materially decreased by food or fasting. Age according to DuBois exercises no great influence on water evaporation for each unit of weight.

**LOSS OF WATER BY THE SKIN.** Water is lost by the skin as insensible and perceptible sweat. The former is continuous as evidenced by the continuous excretion of salt by the skin (Cramer). Sweating is invoked under high environmental temperature around 37°C., but the exact level is influenced by relative humidity, exercise, and certain nervous influences. Loss of water from the skin by transpiration is said to be due purely to physical processes. But this might be questioned on the basis of Talbert's work which demonstrated that volatile acids are excreted continuously during this process. But in patients supposedly devoid of sweat glands losses from the skin up to 800 grams a day have been demonstrated.

*Quantity of water lost by way of the skin.* Under normal conditions of temperature and humidity this is approximately 500 cc. a day. Nuttall credits Pettenkofer and Voit with 500 cc. Obviously this varies markedly with temperature, humidity, winds, exercise, and clothing. Sweat may vary from an insensible perspiration to as much as 1 litre an hour (Haldane and Priestley; White). According to Flack and Hill as much as 10 litres of water (5800 calories) may be evaporated during

a ride in the sun in the South California desert, where the radiant energy reflected from the sand adds its effect to that of the direct radiant energy of the sun.

Water is lost by way of the skin in the absence of sweat glands. Such cases have been described by Guilford, Tendlau, Loewy and Weckselbaum, Christ, and Goeckerman. Loewy and Weckselbaum studied the amount and the mechanism of water lost as insensible perspiration and found in three patients that the amount was normal, 800 cc. each day. Tendlau demonstrated in his patient inefficiency in the heat regulating mechanism, an increase in temperature following the ingestion of milk or direct exposure of the body to the rays of the sun. This patient was studied also by Zuntz who noted increased rate and depth of respiration at all times. In order to work effectively in the sun-light the patient found it necessary to moisten his shirt with water.

*Temperature and onset of sweating.* Visible sweating appears usually between 30° to 37°C. Von Willebrand observed it in one subject at 30°C. and in another at 33.5°C. Subsequent to the appearance of sweat the local temperature may decrease. "The temperature of the naked human skin," says Aron, "if exposed to the sun, rises quickly to 36°C., maximum 37°C. Sweat breaks out then and the temperature falls." For example, the skin surface temperature of the forehead may rise to 41°C. and sink to 35°C.

*Influence of relative humidity.* Von Willebrand found that with a relative humidity of 40 to 50 per cent and a temperature of 12°C., 10.5 grams of water an hour were lost by the skin; at 18°C., 13 to 18 grams and at 28°C., 26 to 27 grams. He believes that the amount vaporized gradually mounts with temperature increasing above 12°C. until sweating begins.

For average clothed persons resting in calm air, a temperature of 25° to 26°C. with a relative humidity of 60 per cent occasions sweating. With 22 per cent relative humidity, the sweating point is nearly 30°C. (Rubner).

The skin varies in its local temperature, its sweating capacity, and its sensitiveness to heat and cold. Thus Benedict found that the skin temperature varied from 28.1 in the calf of the leg to 34.7 in the waist (with clothing). In an artist's model, after 2½ hours' exposure (naked), in a room at 14.5°C., there was a difference of 10.6°C., between the highest and lowest parts; at 25.8°C. the difference was 5.4°C.; at 30°C., 4.2°C. The forehead secretes earlier than the arm and its temperature falls

earlier and generally lower. According to Loewy, the loss of perspiration for each square metre of surface is greatest from the arms, next greatest from the legs (the extremities yielding not far from 75 per cent of the total), and least from the trunk. The greatest actual loss, however, is from the legs (Lusk).

*Temperature and exercise.* The effect of a warm and a cold day on the sweating of marching soldiers as the result of a 7-mile march carried out by four soldiers is shown (Pembrey).

AVERAGE INCREASE OF	WARM DAY	COLD DAY
Pulse.....	6.2 each minute	14 each minute
Rectal temperature.....	2.52°C.	1.44°C.
Evaporation of sweat.....	1816 grams	419 grams
Weight of clothes (moisture).....	320 grams	27 grams

In the summer a young soldier observed by Zuntz, carrying out six marches of 25 km. each, lost between 2436 (1413 calories) and 3366 grams of water (1952 calories), 335 to 995 grams being retained in his clothes.

According to Zuntz, if the body heat production of a soldier is increased 1000 calories by a march carried out at a temperature of 10°C. (50°F.) in saturated calm air, his water loss will be increased 800 grams; each increase of air temperature of 1°C. will increase the water loss 38 grams; each 1 per cent increase of relative humidity will lessen it 4 grams; and each unit increase of wind (12 unit scale of wind) will lessen it 70 grams.

*Sweat and clothing.* Clothing influences water loss by the skin but this will not be discussed.

*Sweat.* Sweat contains from 97.5 to 99.5 per cent water, and is probably the most dilute secretion encountered in the organism. The lack of concentrating power of the sweat glands is in marked contrast to the concentrating power of the kidneys. Perspiration has an acid reaction,  $\text{pH} = \pm 5.7$  (Talbert) and contains, aside from the admixtures of secretions of the sebaceous glands, inorganic salts, chiefly chlorids, and traces of phosphates and sulphates (Kast), and organic compounds of which urea constitutes more than 50 per cent, with smaller amounts of urates, creatinin and etherial sulphates, amino acids, and traces of other metabolic products. The amount of sweat excreted each day varies tremendously with habits, habitat, and climate.

In moderate climates on a daily intake of 3 litres of water the daily loss by sweating during rest approximates 700 cc. for an individual weighing 70 kgm. Heat and exercise markedly increase the amount of sweat. Cramer has estimated from the sodium chlorid left on the surface of the body that more than 3200 cc. are lost daily during a strenuous march in summer weather. By means of dry heat, sweat baths, alcohol or electric cabinets, and so forth, as much as from 0.5 to 1 litre may be lost. Haldane and Priestley refer to two patients who in a Turkish bath, suffered losses as great as 2.3 kgm. of sweat in  $2\frac{1}{2}$  hours.

*The function of sweat.* Sweat, through furnishing water for evaporation, regulates body temperature. This is its chief function. But the sweat glands must also be looked on as excretory organs for water, sodium chlorid, urea and possibly acid radicals. Sweat is a true secretory product and results from the excitation of secretory nerves and is relatively much more independent of blood flow and blood pressure than is the urine. Sweat glands are found everywhere in the skin of man and are very numerous. Cramer has estimated that as many as from 500 to 1900 exist to 1 sq. cm. in some areas of the body.

*Sweat in various animals.* Men and horses sweat from the entire surface of the body. Dogs and cats show visible sweat only from the hairless surfaces, the soles of the feet; while rabbits, mice and rats do not sweat at all. It is usually contended that dogs do not sweat because they have no sweat glands, but cats and dogs do have such glands in other surfaces than the soles of the feet.

*Innervation of sweat glands.* The secretory nerves of the sweat glands belong exclusively to the sympathetic nervous system (Langley). In the cat, for instance, the secretory nerves of the sweat glands of the fore-legs leave the cord with the fourth to the ninth thoracic nerves; and of the hind legs with the twelfth and thirteenth dorsal and first and second lumbar. They all pass through the sympathetic trunk and then through the brachial or sciatic plexus to the balls of the feet.

The spinal centers are controlled primarily by the thermo-regulatory centers in the mid brain<sup>8</sup> but may also be influenced by other parts of the central nervous system and may be stimulated by various sensory stimuli which often produce sweat only in certain limited portions, as for example, the localized sweating over constantly acting muscles (Meyer and Gottlieb). The sweat during nausea and that due to the stimulation of the cerebral cortex from anxiety or fear are familiar examples

<sup>8</sup> The existence of a thermo-regulatory center as such has not been proved.

of the influence of higher centers of the cerebral nervous system (Winkler).

Heat constitutes the most important and most effective physiologic stimulus for the secretion of sweat and acts upon the higher centers. Sweating is invoked by preventing heat loss and increasing heat production, but as a rule it is evoked most readily by the application of heat. Availability of water for excretion in sweat is an important factor in determining the quantitative response. Local heat renders the secreting glands more amenable to stimulation (Schierbeck), while cooling may prevent it entirely (Langley).

*Sweating from drugs.* Sweating may be affected by drugs acting centrally or peripherally. All drugs stimulating spinal centers tend to stimulate spinal sweat centers; thus strychnin, camphor, picrotoxin, pilocarpin and ammonium salts increase sweating, but this power is lost after section of the spinal nerves.

Although the innervation of sweat glands, so far as is known, is purely sympathetic (thoracic autonomic), in their pharmacologic reactions, in their insusceptibility to epinephrin and their susceptibility to the autonomic drugs, they behave entirely like organs with autonomic (parasympathetic) innervation. No explanation has thus far been found for the striking exception to otherwise apparently general laws.

*Induced sweating by high temperatures.* Therapeutically sweating may be induced through the use of high temperatures, either dry or moist, Russian or Turkish baths, dry or moist packs, electric cabinets, hot baths, flannel blankets, and so forth. White found that a few minutes' immersion in a bath at 42.4°C. increased the evaporation of water from his skin eighty times.

**LOSS OF WATER BY THE LUNGS.** Loss of fluid by way of the lungs is continuous and probably not subject to so great variation as that through the skin. Under ordinary conditions the mucous membrane of the mouth and nasopharynx is always distinctly wet with secretions. The air leaves the respiratory tract saturated with moisture and approximately at body temperature.<sup>9</sup> The mucous membrane of the respiratory tract is susceptible to great changes in regard to turgescence and amount of secretions, as evidenced by the marked sensation of dryness in a hot, dry room.

<sup>9</sup> This question is discussed in papers by Galeotti and Osborne. The latter claims that the expired air is saturated at temperatures from 32.5 to 33.5°C.

For quantitative measurements of water loss, air may be collected by special devices (Rubner, Zuntz) in the way of cabinets, chambers, or hoods connected in an air-tight manner with the respiratory tract. Rubner found in the resting man at 20°C. that approximately 400 cc. of water, 17 grams an hour (232 calories), escaped through the respiratory channels which is somewhat less than 50 per cent of the total transpiration.

*Amount of water lost by the lungs.* Benedict and Carpenter, utilizing the computation of Zuntz, found, in a large series of determinations on fifty-three individuals, that the average amount lost by the lungs was 36.3 per cent of the total, with variations from 21 to 51.4 per cent. In sleep the percentage of loss was about 35 per cent.

With the average mean temperature and humidity, Rubner says that an adult gives off water each hour from the lungs: resting, 17 grams; deep breathing, 19 grams; reading, 28 grams; and singing, 34 grams.

The amount of saliva under varying conditions has been studied by Cannon in connection with thirst. Marked effects are demonstrated from mouth breathing, gum chewing, deprivation of water and its administration.

Hill asserts that the water lost during each day by evaporation from the air passages by the resting man can be calculated if the temperature and pressure of the air are taken and the dew point is determined by wet and dry bulb readings.

In the dog the upper respiratory tract is the chief channel of water loss by transpiration. Its influence is indicated by the results following tracheotomy. The rectal temperature of tracheotomized dogs placed in the sunshine rose from 39°C. to 42 and 44°C. The respiration and pulse rate were markedly accelerated, saliva dropped from the mouth, and the mucous membrane became cyanosed.

**LOSS OF WATER BY THE KIDNEYS.** *The urine.* Usually the urine is regarded as representing the major part of the water loss from the body, and, generally speaking, this is true. However, under unusual conditions of heat and exercise water loss through sweat and evaporation from the skin and lungs may far exceed that of the urine.

Urine represents the end result of the work of the kidney. The mechanism involved in the secretion of urine is not known exactly. The well known theories have recently been discussed by Stieglitz who has added some new evidence relative to the functions of the tubules.

The kidney does not pass substances on merely, for in the excretion marked changes occur in relation to concentration. The comparison of the concentration in some of the more important constituents of the urine and blood are tabulated by Cushny as follows:

TABLE 7

	BLOOD PLASMA	URINE	CHANGE IN CONCENTRATION IN KIDNEY
	<i>per cent</i>	<i>per cent</i>	
Water.....	90 to 93	95	
Proteins, fats, and other colloids.....	7 to 9		
Dextrose.....	0.1		
Urea.....	0.03	2	60
Uric acid.....	0.002	0.05	25
Sodium.....	0.32	0.35	1
Potassium.....	0.02	0.15	7
Ammonium.....	0.001	0.04	40
Calcium.....	0.008	0.015	2
Magnesium.....	0.0025	0.006	2
Chlorids.....	0.37	0.6	2
Phosphates.....	0.009	0.27	30
Sulfates.....	0.003	0.18	60

Cushny's idea of the nature of urine in diuresis should be presented. "The urine in diuresis always approaches plasma in composition more nearly than when the secretion is more moderate." In short all the constituents of the urine are increased in absolute amount for each unit of time during diuresis. But the "no threshold" substances are invariably reduced in percentage, while the "threshold" substances are often reduced in percentage, but may actually rise in some circumstances. As the diuresis passes off, a change in the opposite direction sets in.

*The quantity of urine.* Generally speaking, the amount of urine excreted is directly dependent on the water intake and inversely proportional to the amount excreted by the other channels of water loss. Ordinarily the kidney excretes daily an amount of water equal to the difference between the intake and that excreted by the skin, bowels, and lungs when the body weight is maintained at a normal level. This amount fluctuates tremendously, in fact, the ability of the kidney to accommodate itself to fluctuations in water and food intake and to environmental influences, constitutes one of its striking characteristics.

This is utilized clinically in determining renal functional capacity.<sup>10</sup> The normal kidney is capable of excreting a small quantity, from 500 to 600 cc. of concentrated urine with a specific gravity of 1.040 or more, or 8 to 10 litres of very dilute urine with specific gravity of 1.001 to 1.002.

Certain great factors are important in determining the daily output of urine. Among these should be considered age, the amount lost by other channels, food intake, salt intake, and finally and most important, the water intake itself.

*Age.* The urinary output in the early years of life is indicated in Holt's table, his figures being compiled from the findings of several investigators:

<i>Age</i>	<i>Urine gm.</i>
First twenty-four hours.....	0.60
Second twenty-four hours.....	10 to 90
Three to six days.....	90 to 250
Seven days to two months.....	150 to 400
Two to six months.....	210 to 500
Six months to two years.....	500 to 800
Five years to eight years.....	600 to 1200
Eight years to fourteen years.....	1000 to 1500

Camerer gives findings for the daily urine excretion of boys and girls as follows:

<i>Girls</i>					
<i>Years</i> .....	2 to 4	5 to 7	8 to 10	11 to 14	15 to 18 21 to 24
<i>Output</i> .....	670	800	980	930	920 1110
<i>Boys</i>					
<i>Years</i> .....	5 to 6	7 to 10	11 to 14	15 to 16	17 to 18
<i>Output</i> .....	730	940	1040	840	1040

The number of voidings and the average quantity of each is shown in table 8.

The rate and amounts of urine excretion following the intake of definite amounts of water can be found in the papers from Aschenheim and Ohlmann.<sup>11</sup>

*Adults.* The water intake varies markedly from day to day. The average according to Forster is from 2300 to 3500 cc. These figures are from Munich and include consumption of beer. For this country,

<sup>10</sup> For details concerning concentration and dilution tests see Volhard and Fahr.

<sup>11</sup> For details concerning urinary output in childhood see Feldman.

Atwater and Benedict found variations in water intake from 880 to 2440 cc. with the subject in repose, an average for 49 days of 2290 cc. and with moderate work from 2225 to 4550 cc., an average for 66 days of 3700 cc. The urinary output varies with the intake within normal limits from 800 to 3000 cc., the average being usually between 1200 to 2000 cc.

TABLE 8

AGE		MICTURITIONS*	AVERAGE QUANTITY VOIDED AT EACH MICTURITION cc.
Days,	14 to 30.....	13.0	34
Months,	1 to 3.....	14.0	31
	3 to 6.....	20.0	31
	6 to 12.....	16.0	44
Years,	1 to 2.....	12.0	60
	2 to 3.....	10.0	88
	3 to 4.....	9.0	92
	4 to 5.....	7.5	90
	5 to 6.....	9.3	104
	6 to 7.....	7.1	154
	7 to 8.....	7.8	146
	8 to 9.....	7.0	191
	9 to 10.....	7.3	262
	10 to 11.....	7.0	248
	11 to 12.....	7.5	224
	12 to 13.....	8.3	262

\* According to the investigations of Engel and Pfeifer the number of micturations during the first few months of life may be much greater.

*The effects of environment.* Other things being equal, the quantity of urine varies directly with the water ingested and inversely with the loss from the lungs and skin. During extreme hot weather, the water intake may be very large and the urine output may be quite small. Breinl and Young have contrasted the urine output in the tropics with that of the European standard. The volume of urine excreted is smaller, the specific gravity higher, and the total nitrogen and sodium chlorid content less in the tropics, as shown by the table giving the average of twenty-five persons some of whom were manual and some sedentary workers:

	IN TOWNSVILLE,* AUSTRALIA	EUROPEAN STANDARD
Quantity.....	782 cc.	1500 cc.
Specific gravity.....	1.025	1.015 to 1.020
Freezing point $\Delta$ .....	0.935 to 2.256°C.	0.87 to 2.71°C.
Total nitrogen.....	10.4 gm.	16.0 gm.
Sodium chlorid.....	7.0 gm.	15.0 gm.
Phosphates.....	1.73 gm.	2.0 to 3.5 gm.

\* Townsville, Australia, has a latitude of 20 S. and longitude of 147 W.

In hot climates the water balance is usually set at a high level. Hunt says that the fluid intake and output in India is commonly as much as 13 litres each day. In temperate zones, there is a striking difference in the urinary output in summer as compared with winter.

*Food and urinary output.* Food influences water exchange at least in two important respects, the effects of the metabolites on the water holding capacity of the body, osmotic pressure, salt content, and so forth, and the effects of metabolites on renal function.

Reference has already been made to the effects of diets rich in carbohydrates or fats on body weight and on water output. Rubner calculated the amount of water necessary in tropical weather to yield sufficient water to maintain a properly dilute urine and sufficient sweat to keep the individual cool. This varies with diet. On European diet, 2400 calories a day, 4400 cc. of water are required. On an exclusive meat diet, 7600 cc. of water are required. The effect of types of food on the urinary output is striking. Doctor Adams, of our staff, is studying the effect of diet on the quantity of urine. On each of three days, the patient was given 700 cc. of water with breakfasts rich in carbohydrates (dextrose 1.5 grams for each kilogram of body weight), protein, or fat respectively. On the breakfast high in fat, 27 per cent of the water was recovered in urine within 5 hours, on the diet high in protein, 74 per cent, and on the diet high in carbohydrates, 85 per cent.

Obesity markedly affects water exchange. Oertel's researches show that the quantity of water entering the organism affects the accumulation and consumption of fat. When the water loss is great and the intake small the fat accumulated in the body decreases. Beeler and Fitz have demonstrated however that in one form of obesity, diuresis is induced only with the greatest difficulty. But, in general, it is only with the greatest difficulty that water is held in the body. Accord-

ing to Schattenfroh, the loss of water by transpiration, occurring in fat persons during exercise, is greater than in thin. Lack of training probably plays some part.

Excessive ingestion of sodium chlorid and of sugar results in increased thirst, increased water ingestion, and increased urinary secretion. The ingestion of large quantities of hypertonic salt or sugar solution, results in extreme diuresis; water withdrawal and failure to replenish the excessive loss results in dehydration of the body and development of fever.

*Beverages.* In addition to the diuretic effect of the contained water, many beverages directly stimulate the renal secretion. Thus the xanthin derivatives in tea, coffee, and cocoa exert a specific stimulating effect on the cells of the renal tubules (Schroeder); and an increased blood flow through the kidney (Loewi). Alcohol, under certain conditions, causes vasodilatation of the kidney and diuresis. Beer is especially effective in this respect.

*Control of urinary secretion.* Embryologically and histologically, the kidney is not a typical secreting gland. It consists of a large number of units, tubules and glomeruli, and has a relatively large blood supply. The kidney presents within itself the same double capillary arrangement encountered in the portal system; the glomeruli corresponding to vessels of the alimentary tract and the second series around the tubules corresponding to the capillaries of the liver.

The number of units, glomeruli and tubules, is large. In the dog, various investigators have placed the number at from 125,000 to 300,000 depending on the size of the animal (Brodie and Peter); in the cat, 16,000 (Miller and Carlton); in man, 2,000,000 (Schweizer-Seidl). The number is generally in excess of the actual needs, that is, the factor of safety is great as evidenced by the fact that one-third of the kidney tissue will suffice.

The kidney derives its nerve supply from *a*, the semilunar ganglion; *b*, a small branch direct from the splanchnic; *c*, a small branch from the plexuses around the suprarenal body and aorta; and *d*, sometimes a direct branch from the vagus. The splanchnic nerves carry vasoconstrictor fibers. Stimulation results in the arrest of renal secretions (Bradford) and section, in increased flow (Claude Bernard). The vagus, through its action on the heart, exercises a marked influence on renal secretion. Its stimulation in the neck causes a marked decrease of urinary flow, but the presence of fibers directly affecting renal secretions has not been proved. The nerve control is exercised ordinarily through

the vasomotor center in the medulla. This may be stimulated directly by asphyxia or anemia of the medulla oblongata, resulting in decrease or arrest of urinary secretion from ischemia of the kidney, or reflexly through the sensory nerves such as the sciatic (Cohnheim and Roy), through cutaneous nerves as in exposure to cold, or through stimuli arising from within the kidney, ureter, or bladder. But it must be remembered that the kidney can function for months in the absence of all nerve supply, as has been shown by Carrel and Guthrie, and Quinby.

Cerebral control is exercised also. The details as to the nature of the mechanism are still lacking. The results of puncture in the floor of the fourth ventricle, Claude Bernard's *piqûre* (Bernard, Eckhard and Finkelnburg) are familiar. Brain tumors are often accompanied by urinary disturbances, especially those which involve the pituitary gland or its neighborhood. The work of Camus and Roussy indicates that independent of any injury to the pituitary gland, polyuria results from the puncture of the interpeduncular space, and that of Bailey and Bremer indicates that polyuria constantly follows a *piqûre* injuring the para-infundibular region of the hypothalamus. Polyuria associated with or following epilepsy, migraine, or other headaches, hysteria, and nervous strains suggests strongly the existence of higher centers of control.

The endocrine system also unquestionably plays a part in the control of water balance. This is indicated by the striking effect of the subcutaneous administration of the extract of the posterior lobe of the pituitary in controlling the urinary output, in normal individuals following excessive water ingestion and in patients with diabetes insipidus. Also polyuria results often following extirpation of the posterior lobe of the pituitary or section of the infundibulum. According to Cushing, the pituitary is involved in the control of urinary output through nerve tracts reaching the kidney after passing from the spinal cord to the superior cervical ganglion and posterior ganglionic fibers. Similarly, the thyroid gland is concerned in the metabolism of water as evidenced by changes wrought in a myxedematous patient. On the administration of thyroxin the patient quickly loses weight—largely water—and the dry indurated skin becomes soft and moist.

*Some physiologic factors influencing urinary secretion*, such as blood flow, blood pressure, etc., will not be discussed.

*Physico-chemical factors.* The paramount influence in urinary secretion is the chemical composition and physio-chemical state of the blood. Two factors are of great importance, changes in concentration

of the colloids of the plasma and permeability of the kidney for substances appearing in the plasma. Decreased colloid content tends to increase urinary output, that is, to "dilution diuresis." The dilution of colloids is most readily accomplished by the intravenous injection of salt solution or Ringer's solution. Such a procedure usually results in calling into play mechanical features, as is evidenced by the increase in the volume of the kidney as recorded by the oncometer. But this does not invariably result (Starling, Cushny). Richards and Plant have shown that the saline diuresis may occur without increase in renal volume or in blood pressure.

Magnus, who conducted classical experiments in salt diuresis, first concluded that it was due to hydremia, but later withdrew this conclusion and ascribed it to specific stimulation of renal cells. In a critical review of the experiments of Magnus, Cushny has reinterpreted his results and finds that decrease in colloid contents of plasma accompanies all instances of diuresis.

EXPERIMENT	NORMAL		DURING DIURESIS	
	Urine in 10 minutes	Plasma-protein	Urine in 10 minutes	Plasma-protein
	cc.	per cent	cc.	per cent
1	1.2	7.33	115.0	3.84
2	1.0	5.94	57.5	3.17
3	1.5	4.43	62.5	2.86

In some experiments when the colloids of the blood are decreased by less than one-half, the urine is increased a hundred fold. Cushny explains this on the basis that the rapid flow of urine through the tubules, that is, flooding, prevents reabsorption and consequently only a small proportion of the excess passing through the glomeruli is reabsorbed, the greater bulk of urine reaching the ureter before absorption. The work of Barcroft and Straub lends support to the idea that lessening the osmotic resistance in filtration is responsible for diuresis, in that the oxygen consumed during the period of diuresis does not exceed the normal amount for the resting kidney. The volume of blood is not responsible, since replacement of colloid by salt solution results in marked diuresis. Knowlton has brought convincing proof, by injecting saline solution with and without colloids and finding that the diuresis is proportional to the free saline solution. Fischer has pointed out that availability of water is important in relation to its excretion and has emphasized the importance of considering the factors affecting the

hydration properties of the body tissues. "Free" water only is available for excretion. Meyer and Gottlieb have found that by diminishing colloid content of the blood, urinary secretion can be obtained at as low a pressure as 13 mm. of mercury.

*Sodium chlorid diuresis.* When sodium chlorid is taken by mouth in excess it may be excreted *a*, in increased concentration but without diuresis; *b*, in normal concentration with diuresis; or *c*, with decreased concentration and diuresis. What happens probably depends on the concentration, at the time, of sodium chlorid and the amount of water in the blood and tissues of the organism. The concentration of other substances is also of importance.

The response to salt intake is extremely important in certain types of nephritis in which the salt and water content of tissues is already disturbed. Additional salt under these conditions usually leads to dropsy and control of salt intake is important in the control of dropsy (Widal and Javal). The recent work of Haldane and Priestley is important in that it shows that water taken by mouth in excess does not result in demonstrable dilution of the blood, whereas following the ingestion of the same quantity of Ringer's solution dilution can be shown.<sup>12</sup>

*Water diuresis.* Distilled water administered intravenously does not lead to immediate diuresis (Thompson). It quickly leaves the blood stream, owing to the higher osmotic pressure of the cells of the body, which causes it to be taken up by the tissues. Larger quantities leave the blood. Similarly, subcutaneous injection is not followed by diuresis for the same reason (Ginsburg and Cow). Polyuria usually appears within a few hours following the ingestion of water by mouth in excess of from 10 to 15 cc. for each kilogram (Hashimoto).

As already indicated, the most important factor affecting the urinary output is the intake of water. When large quantities of water are ingested by mouth, profuse excretion of very dilute urine follows. As much may be passed in an hour as is ordinarily voided in 24 hours. How is this effected? No adequate explanation has been given. The inference is that water is absorbed; the absorption results in hydremia, which in turn results in diuresis. This appears rational at least, but Haldane and Priestley failed to demonstrate any change in the concentration of the hemoglobin of the blood following the ingestion of water up to 5.5 litres in 6 hours, which resulted in a urinary secretion as high as 1200 cc. in 1 hour, probably the greatest diuresis ever observed in man

<sup>12</sup> Ringer's solution practically speaking is optimal fluid.

under normal conditions. They conclude that no demonstrable dilution of the blood accompanied the marked diuresis occurring after the ingestion of huge quantities of water. Priestley, however, in other experiments, succeeded in demonstrating a very slight decrease in the electrical conductivity of the blood and believes that the slight diminution in electrical conductivity indicates very slight decrease in salt concentration of the blood and that the kidney is extraordinarily sensitive to changes in concentration of the blood. He likens the response of the kidney to slight dilution of the blood to the marked increase in ventilation of the lungs resulting from extremely small changes in the carbon dioxide content of the blood. This may be so; however, it has not been proved as yet. Fischer explains diuresis as a result of an excess of free water being brought to the kidney. Cow, failing to find any adequate explanation for the differences in the diuresis from water, when it is given hypodermically and by mouth, has suggested that in the latter case an enzyme from the alimentary tract comes into play. Adolph believes that water held by the tissues is distinct from water freely absorbed from the alimentary tract. Whatever the mechanism may be, this polyuria can be prevented by the administration subcutaneously of a small amount of the extract of the posterior lobe of the hypophysis.

In diuresis from water ingestion the percentage of urinary solids is largely reduced but the absolute quantities over short periods of time and usually the total excretion tend to increase. The various solids however, do not increase in the same proportion (MacCallum and Benson, Marshall, Carr).

The secretion of urine more dilute than plasma is another matter difficult of explanation. This fact is constantly utilized to disprove Ludwig's theory of urinary secretion and to support the idea of specific secretion of urine. It is difficult to conceive of a urine more dilute than glomerular filtrate (deproteinized plasma) resulting from a mechanism involving filtration of deproteinized plasma and subsequent reabsorption of Ringer's solution by the tubules. Burian believes that the glomeruli secrete a very dilute solution after large amounts of water and Frey that the glomerular solution is unchanged but that the tubules secrete water in addition. Cushny, however, believes that the explanation is not difficult. He states that water is absorbed, enters the blood as a dilute saline solution, and is rapidly carried to the kidneys "where it is filtered off in a form slightly more dilute than the optimal fluid; that the subtraction of the latter in the tubules leaves water containing

urea and the other 'no threshold' substances and that such 'threshold' bodies as are above the threshold, potassium, uric acid and almost all of the chlorid, are reabsorbed in the fluid, but some escape." He illustrates this by a hypothetical example, which, however, involves a 10 per cent dilution of plasma which he admits is far beyond the actual dilution ever encountered in the plasma. Thus it is seen that much concerning renal secretion still awaits solution.

Explanations involving the physiology of ordinary diuresis from water must precede those dealing with abnormal or pathologic forms of diuresis. MacCallum has obtained, in man, a diuresis of 20 cc. a minute; Priestley, one at the rate of 1200 cc. an hour from water. Priestley intimates that this rate of elimination results in strain and consequently cannot be sustained. Yet Trousseau records a case of diabetes insipidus in which the patient voided 43 litres of urine a day. Enormous quantities of urine, 15 to 25 litres a day, may be excreted for days, weeks, months or years by persons suffering from this disease without the manifestation of fatigue on the part of the kidney, and without evidence of renal insufficiency. In the primary type of this disease, pathologic conditions cannot be demonstrated. Attempts to demonstrate dilution of plasma by cryoscopy have yielded discordant results,<sup>13</sup> as have also attempts to show increase in the volume of blood (Larson, Weir, and Rowntree). Consequently, we are forced to the conclusion that no adequate explanation has been given for diuresis following the drinking of water, for the polyuria of the diabetes insipidus, or for the antidiuretic effect of pituitary extract.

*Water in the feces.* Under ordinary conditions the water of the feces rarely amounts to more than 200 cc. in health. Usually it is between 60 and 150 cc. On a vegetarian diet it may reach 300 cc. a day. Occasionally attacks of diarrhea develop, for a day or so, resulting in a doubling or trebling of this amount. But on the whole in health the amount is small and fairly constant.

*Water requirements of the body.* For continuous health the water intake of the body must suffice to keep the water content at the level for maximum efficiency, from the physiologic viewpoint, irrespective of the loss of water by the various channels of excretion. The important determinants have been presented. This necessitates an intake large enough to

<sup>13</sup> It is possible that pituitary extract changes the relative ease with which water passes from the blood stream to the tissues, on the one hand, or to the kidneys on the other, or that it affects the rate of its reabsorption in the tubules of the kidney.

replenish the store as losses occur. Additional water is required during childhood for the building up of tissues. Its importance in infancy can be seen from the following data from Reusing.

On the breast, the intake the first day is 38 cc. and the urinary output 8.4 cc., while on the eighth day the intake is 338 cc. and the output 208 cc. In artificial feeding, the water intake is usually larger, the amount the first day being 96 cc. and the urinary output 36 cc.; the intake on the eighth day is 530 cc. and the output 406 cc.

There still remains for consideration the effects on the organism of water restriction or deprivation, of water withdrawal from the tissues, and of excessive water ingestion.

**THE EFFECTS OF WATER DEPRIVATION.** As has been intimated, the body need of water is indicated by thirst. According to McGee, thirst may be divided into five stages:

1. The mouth and throat become dry; a longing for liquid is easily assuaged by ingestion of fluids.

2. The saliva and mucus in the mouth and throat become scant and sticky; the tongue clings to the teeth or to the roof of the mouth; there is a lump in the throat and endless swallowing; this stage is also greatly relieved by water.

3. The eye lids stiffen over the eyeballs which set in a sightless stare.

4. The distal end of the tongue hardens to a dull weight.

5. Delirium develops with visual illusions of lakes and running streams.

Dryness of the mouth is very striking. Reference has been made to King's report on the degree of dryness of the mouth. Suffering was intense and those who survived did so by drinking their own urine or horses' blood.

Thirst is more difficult to endure than hunger. Viterbi, an Italian political prisoner, who died as a result of refraining from food and water for 18 days, suffered but little from hunger after the first day but experienced terrible thirst until the end.<sup>14</sup>

The period that life can be tolerated without water varies tremendously, depending largely on environmental conditions and muscular activity. Thus in the desert, when evaporation is extreme, death occurs as a rule in from 36 to 72 hours. An instance is described in which a Mexican, lost in the desert, walked and crawled 150 miles

<sup>14</sup> According to Hertz, *The Sensibility of the Alimentary Canal*, London, 1911, there comes a time (case quoted) at which neither thirst nor hunger causes distress.

for a period of 7 days. He sustained life in part by drinking his own secretions.

Animals have borne complete starvation from water and food as long as 28 days with recovery. One of Poletayeff's dogs survived 22 days without water with 47 per cent loss of weight, and succumbed in a subsequent starvation only after a loss of 60 per cent body weight. At necropsy, fat was still found in the neck and in the abdominal wall and cavity. A rabbit survived 8 days' complete starvation, and lost 32 per cent body weight. After 7 days it was again starved, and died on the tenth day with 40 per cent loss of weight. Groll has shown that with complete starvation the hemoglobin may increase prior to death 13 per cent in rabbits, 28 per cent in cats, and 18 per cent in dogs.

Keith deprived dogs of water and food over periods of from 2 to 4 weeks. Blood and plasma estimations on these animals showed a definite decrease in the amount of circulating blood. Associated with this diminished blood volume there was an increased viscosity of the whole blood. The viscosity of the plasma may or may not be increased. Partial restoration of blood volume followed the giving of water by mouth. With the addition of food to the water ration the volume of blood appeared to increase more steadily. With this partial restoration of the blood volume, there was a dilution of the previous concentrated blood which was evidenced by a lower viscosity and a decrease in the concentration of the chlorid and nitrogen in the plasma. Some interesting changes were also noted in the hemoglobin and red cell content of the blood during these striking volume changes.

*Withdrawal of water from the tissues.* In 1899, Crandall described febrile attacks in infancy, occurring in inanition, which he showed to be due to thirst and which disappeared on the administration of water. Finkelstein, in 1908, described fever in children resulting from the administration of hypertonic lactose solution (12.5 per cent) and also from salt and glucose administration, conditions which have since been referred to as salt or sugar fevers. Subsequent workers, Heim and John and Peteri, interpret salt fever as resulting from desiccation and decreased evaporation of water due to the hydropigenous or edema producing action of salt in the blood. Peteri found that the height of the fever attained was in inverse ratio to the body weight. Woodyatt and his collaborators have succeeded in producing glucose fever in adult humans and in dogs through the intravenous administration of concentrated solutions of glucose. In man, difficulty was encountered in eliciting the hyperthermia with single doses of glucose. But they

determined that it could be produced readily, provided a period of starvation preceded the glucose administration.

Woodyatt, utilizing his method of constant rate of injection, has produced fever up to 125.6°F. with glucose and up to 111°F. with salt and lactose. He ascribes the hyperthermia to water deprivation, since he has prevented fever by administering water in experiments in which he gave 10 grams an hour for each kilogram for a period of 7 hours. By producing fever with crystalloids, he excluded combustion of sugar

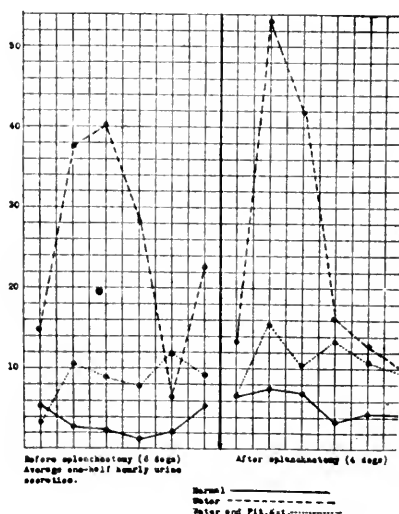


Fig. 2. Average half-hour rate of secretion of urine in dogs under normal conditions, after water, and after water and pituitary extract: (a) before section of the renal nerves (six dogs) and (b) after section of the renal nerves (four dogs).

as the cause; by preventing convulsions with anesthesia, he excluded convulsions as the cause; and by producing fever in dogs rendered poikilothermic, he excluded action on the heat centers as the cause. Accepting Fischer's conception of "bound" and "free" water, he holds that generally purely physico-chemical processes rob the tissues of free water and disturb the equilibrium between free and bound water. Similarly, he feels that febrile diseases such as typhoid fever and pneumonia may well be the result of free water deficit, due to the abnormal binding capacity of the colloids, and cites the familiar diuresis accompanying the crisis of pneumonia or edema as disturbed water

metabolism. He looks on fever, as Fischer does on edema, as due to physico-chemical changes in colloids resulting in abnormal water binding. Barr and DuBois have pointed out that the relationship between the percentage of calories lost through the vaporization of water and the total heat elimination is the same in patients with fever as in normal controls and in patients with various afebrile diseases.

THE EFFECTS OF EXCESSIVE INGESTION: WATER INTOXICATION. Through thirst the intake of water is regulated to the body needs. As a rule, the amount exceeds the absolute need. Unless the intake is greatly in excess, it is readily excreted by the kidneys and in a less amount by the skin.

In diabetes insipidus, the water balance is set at a level higher than normal. The level can be lowered immediately by the subcutaneous administration of the extract of the posterior lobe of the hypophysis (Von der Velden and Farini). Subsequent to the use of the extract the ingestion of water in the large amounts to which the patient has become accustomed results in marked toxicity. Larson, Weir and Rowntree observed patients who developed headaches, nausea, asthenia, incoördination, marked sweating and, in one instance, mild subcutaneous edema under these conditions. These findings are in accord with those of Miller and Williams. Patients with chronic nephritis and hypertension, to whom they administered water up to 10 litres a day, developed headache, dizziness, restlessness, chills, fullness of the abdomen, vomiting, dyspnea and cramps in the legs, marked increase in weight, and increase in blood pressure.

Water intoxication was produced in dogs by Larson, Weir and Rowntree through the administration of large amounts of water by the stomach tube subsequent to subcutaneous administration of extract of the posterior lobe of the pituitary gland sufficient to prevent the development of polyuria.<sup>15</sup> The ingestion of large quantities of water at hourly intervals, 50 cc. for each kilogram, subsequent to 3 cc. pituitary extract, resulted regularly in the development of the following train of

<sup>15</sup> The history of the development of our conceptions relative to the rôle of pituitary extract and the function of the posterior lobe is of singular interest. Schafer and his co-workers called attention to the diuretic properties of the extract of the posterior lobe. The work was conducted on animals under the influence of anesthetics. Following this a chemical conception arose involving diabetes insipidus resulting from underfunction of the pituitary (posterior lobe). Subsequently Von der Velden and Farini independently discovered its anti-diuretic influence in diabetes insipidus. The extract temporarily removes all the cardinal symptoms of this disease.

symptoms: asthenia, restlessness, frequency of urination, diarrhea, nausea, retching, vomiting, tremor, salivation, muscle twitching, ataxia, convulsions tonic and clonic, frothing at the mouth, and stupor or coma. Death ensued when water administration was continued after the onset of the convulsions, whereas complete recovery resulted

TABLE 9  
*Water intoxication*

TIME	WEIGHT	WATER INTAKE	TEMPERA- TURE	REMARKS
Dog ES04				
8:45 a.m.	5.7	300	98.0	(After water starvation for 2 days)
9:15 a.m.		300		
9:45 a.m.		300		
10:15 a.m.		300		
10:45 a.m.		300		
11:15 a.m.		300		Salivation, vomiting
11:45 a.m.	6.2	300	98.0	
12:15 p.m.		300		Twitching, vomiting
12:45 p.m.		300		Ataxic
1:15 p.m.		300		
1:45 p.m.	6.4	300	98.0	Convulsions lasting two minutes
1:50 p.m.				Stomach emptied with tube; about 200 cc. fluid obtained
Rabbit 3				
9:30 a.m.	1.7	100	97.9	Distilled water at body temperature
10:15 a.m.		100		
11:15 a.m.		100		
11:45 a.m.		100		
12:00 m.	2.1			Salivated; ataxic convulsions lasting two minutes
12:15 p.m.	2.1		98.0	
1:05 p.m.	2.1			Found dead

within 12 hours in a large proportion of the animals if no more water was given. Pituitary extract prevents the development of marked diuresis as indicated in the preceding chart.

Rowntree has since succeeded in inducing water intoxication in various animals without the use of pituitary extract. In dogs, the administration by stomach tube of 50 cc. for each kilogram of body

weight at half-hourly intervals results regularly in nausea, vomiting, salivation, convulsions, stupor, and coma within 4 to 8 hours; death ensues if water administration is continued after the onset of convulsions. Cats, rabbits, and guinea pigs are similarly affected by water (table 9).

This intoxication results from ingestion of ordinary drinking water or distilled water, irrespective of temperature. Although the quantities given are excessive, the amount absorbed is definitely limited.<sup>16</sup> The intoxication is not accompanied by significant changes in body temperature, by edema, or by constant or marked increase in plasma volume. The blood pressure is somewhat increased and may reach as high as 200 mm. of mercury during the actual convulsions. Intracerebral pressure in one experiment was found to be increased to an amount corresponding to that of 35 cc. of water, and this increase was not in amount proportional to or parallel to the increase of blood pressure.

The convulsions are cerebral in origin and of extreme violence at times; they last from one to ten or fifteen minutes. They may be tonic but are usually clonic in character and they are apt to recur at intervals. In the interim, the animal presents a state of stupor or coma with marked muscular flaccidity, extreme asthenia, and abject helplessness. Hypertonic salt solution administered intravenously after the onset of early evidences of toxicity, prevents the onset of convulsions and coma. Necropsy reveals no gross changes.

<sup>16</sup> The organism possesses some mechanism whereby it protects itself from too great absorption of water. In numerous animals (dogs, cats, and rabbits), following the administration of large quantities of water by the stomach tube, only relatively small quantities of water were absorbed as indicated by the weight.

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# PHYSIOLOGICAL REVIEWS

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## THE CEREBROSPINAL FLUID

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It is obviously impossible within the limitations of this review to present a truly comprehensive account of a characteristic body-fluid such as the cerebrospinal liquid. During the last century many contributions to our knowledge of this fluid have been made, though rather sporadically and with long intervals between publications. Anatomists, physiologists, pathologists and other workers have studied the problems of this fluid; the proper presentation of the many phases of the subject would lead into all of the representative biological sciences. But during the past two decades contributions of a physiological and anatomical nature have resulted in definite enlargement of our conceptions of this fluid which so completely fills the cerebral ventricles and surrounds the central nervous system. It is largely with these more recent advances in knowledge that this review will deal, taking from the older literature only those contributions which have founded the essential bases of the biological processes of the cerebrospinal fluid. It is purposed to omit in large measure the exact chemical, pathological and serological aspects of the subject except as the data from these investigations aid in establishing the fundamental anatomical and physiological phenomena which have to do with this fluid. For in this problem, as in many others, it has seemed obvious that the furtherance of investigations upon function has depended largely upon equivalent advance in anatomical knowledge.

The cerebrospinal fluid, as first effectively described by Magendie (48), is a clear limpid liquid of low specific gravity (1.004 to 1.006), colorless, and of a slight but definite viscosity. When withdrawn during life, the liquid usually contains but few cells per cubic millimeter (less than 10) but in many pathological conditions its cell-content may be enormously increased. Various estimates of the amount of the fluid existing in the cerebral ventricles and about the nervous system in adult man have been published; the computation of 100 to 150 grams

given by Testut (65) is probably the most reliable, but because of the complexities of the fluid-bed the figures should necessarily be taken as an approximation.

Chemical examinations have demonstrated that the cerebrospinal fluid contains small quantities of inorganic salts, of protein and of dextrose. The inorganic salts are chiefly sodium chloride and potassium chloride; these occur in the ratio of 17.3 to 1, according to Mestrezat (50), whose monograph is a compendium of analyses of both normal and pathological fluids. The average pH value is given by Felton, Hussey and Bayne-Jones (27) as 7.75, with maximum variations of 7.4 and 7.9. Such chemical and physical characters induced Halliburton (37) to term the cerebrospinal fluid "an ideal physiological saline solution," bathing the neurones and maintaining their osmotic equilibrium.

This liquid, then, of distinctive constitution, unlike other body-fluids (except the aqueous humor of the eye) becomes the subject of review. Definite conceptions regarding its circulation are current, for the evidence today points to a constant production of the fluid, its passage through the cerebral ventricles and thence throughout the subarachnoid space, and its subsequent major absorption into the venous system. Necessarily, however, these conceptions must be subjected to critical analysis, to determine the character and reliability of the data supporting these viewpoints. For this purpose it becomes essential to arrive at some understanding of the anatomical mechanisms involved and to ascertain the functional employment of these structures as pathways for the cerebrospinal fluid. Such a discussion, while in no way comprehensively covering the problems of the cerebrospinal fluid, will present at least in part the phases of the subject which have in recent years been most extensively investigated.

#### THE SOURCES OF THE CEREBROSPINAL FLUID

The description of the glandular histological structure of the choroid plexuses by Faivre (25) in 1853 marked the discarding of the older concept of Haller and Magendie that the cerebrospinal fluid was a product of the leptomeninges (particularly of the pia mater). Faivre made the first histological survey of these villous projections, showing that the cell coverings were epithelial in nature and that there were indications of secretory activity in these cells. Faivre's observations, supported by similar histological descriptions of hyaline-like inclusions by Luschka (47) in 1855, gave origin to the hypothesis that the choroid plexuses elaborate the greater portion of the cerebrospinal fluid; this has remained

the hypothesis upon which most of the investigations regarding the source of this peculiar body-fluid have been based.

The purely histological evidence presented in support of this hypothesis, while suggestive, lacks the element of conclusive proof, though for many years accepted without question. During the past twenty years, however, renewed attempts with finer histological and cytological methods have been made to bridge the gap between intracellular secretion-granules (vacuoles) and the actual production of the liquid surrounding the cells. Thus, Findlay's (28) description of the granular structure of the normal epithelial cells of the plexus, with frequent inclusions of slightly pigmented globules representing the fusion of smaller elements and staining with osmic acid, is quite typical of the purely histological demonstration of secretion. Studnicka (64) obtained somewhat similar evidence of secretion by the cells of the plexus and by the ependymal cells of certain areas. Pettit and Girard (55) found hyaline-like globules, similar to those described by Luschka, in the cells of the plexuses in a comprehensive series of animals but did not feel that they represented secretion-vacuoles. Loeper (46), working on the plexuses in man, described pigmented granules and other granules within vacuoles staining with osmic acid; he stated that he believed that such histological findings permitted him to assert that the cells of the choroid plexuses are glandular. Employing methods of supravital staining in addition to the ordinary histological procedures, Schläpfer (61) concluded that the protoplasm of the cells of the choroid plexus contained "globoplasten" surrounded by a lipoid-like capsule; his histological findings offer but little additional support for his assertion that the choroid plexuses secrete the cerebrospinal fluid. Galeotti's (35) description in rabbit and mouse of three different intracellular inclusions (hyaline droplets, fuchsinophilic granules and small basophilic plasmosomes) afforded evidence of cell-activity by the choroidal epithelium but did not demonstrate the elaboration of the liquid by these structures. The same statement may be made of Francini's (32) differentiation of two forms of secretion-phenomena in these cells—droplets formed in the cytoplasm and granules derived from the cell-nucleus. Engel's (23) demonstration of a fuchsinophilic granule and a basophilic granule staining with methyl green is purely histological evidence of intracellular inclusions but the great variability in position of the granules can hardly be interpreted as indicating different phases in the secretion-process. And there is likewise no conclusive proof of function in Hworostuchin's (41) description of the changes in form of the mitochondria of the choroidal epithe-

lism as showing that the cells play an active rôle in the elaboration of cerebrospinal fluid. Yoshimura (80), in a histochemical investigation of the cells of the plexus, believed that the complicated process of secretion of the cerebrospinal fluid was related to the aggregation of the smaller cytoplasmic granules together into larger vacuoles for discharge. Pellizzi's (54) hypothesis that the epithelial cells of the plexuses secreted granules which increased in size by absorption of the fluid-plasm until extruded, was based on a comprehensive study of the plexuses in vertebrates and may likewise be considered as suggestive support of the general thesis.

It seems clear from the observations just detailed that while the many workers upon the histological structure of the choroid plexuses have described certain granules and vacuoles in these epithelial cells, there is no conclusive evidence that these intracellular structures constitute the intracellular mechanism for the elaboration of cerebrospinal fluid. The difficulty of final demonstration that these granules are discharged into the fluid or are in some way dissolved in that fluid, cannot at present be surmounted.

But fortunately observations of a far more conclusive nature have been made by a combination of pharmacological and histological methods. Cappelletti (9) reported in 1900 that ether and pilocarpine increased the flow of cerebrospinal fluid from an experimental fistula and that atropine and hyoseyamine diminished it. While considering that the differences in vascular reaction to these drugs might account for the phenomena observed, Cappelletti felt that the action of pilocarpine as a general stimulant of gland-activity justified the assumption that there was a true acceleration of secretion of the cerebrospinal fluid. Pettit and Girard (55) extended these observations of Cappelletti by introducing histological examinations of the choroid plexuses in animals which had been given muscarin, pilocarpine, ether, theobromine, etc. The administration of these substances was found to increase the volume of the cytoplasm of the choroidal epithelium, so that the cells became doubled in height. Histological study of these enlarged elements, both in the fresh and in fixed material, showed that the cells were divided into a densely granular basilar zone and a clear apical zone. While the clear apical area was indicated in the resting cell, the rapid enlargement of this zone, under the influence of muscarin, ether and theobromine, resulted in the formation of a distal clear vesicular mass. Such a demonstration of histological change in the choroidal epithelium under the influence of drugs which caused an increased flow of cerebrospinal

fluid from a fistula, was believed by Pettit and Girard to prove conclusively that the choroid plexuses possessed a secretory function in the elaboration of cerebrospinal fluid.

Meek (49), repeating the experiments of Pettit and Girard, came to identical conclusions. Muscarin caused no change in the choroidal epithelium in the rat but in the rabbit and guinea pig definite alterations were recorded. Meek stated (p. 300) that "the two things most striking about these modified cells are their great increase in height and the appearance of so much clear space at the distal side of the nucleus."

While the observations just quoted definitely relate the choroid plexuses to the elaboration of cerebrospinal fluid, there is available other substantiating evidence in support of this hypothesis. It had long been realized, from pathological examinations of cases of obstructive internal hydrocephalus, that the cerebrospinal fluid must be at least in part produced by some intraventricular structure. In this relationship renewed attention was directed to the choroid plexuses by the discovery by Claisse and Levy (12) in 1897 of a case of internal hydrocephalus associated with hypertrophy of these intraventricular plexuses. Dandy and Blackfan (17), (18) and Frazier and Peet (33) gave additional support to the general contention when they experimentally produced an internal hydrocephalus by occlusion of the aqueduct of Sylvius. Cushing's (14) observation of an exudation of a clear fluid from a choroid plexus exposed in exploration of a porencephalic defect likewise added suggestive substantiation of the hypothesis. Somewhat more tangible proof of intraventricular elaboration of the fluid was afforded by the writer's (68) demonstration that a definite and sustained outflow of cerebrospinal fluid could be obtained by catheterization of the third ventricle through the aqueduct of Sylvius. The outflow from such a catheter was quite similar in amount to the fluid obtained from a cannula in the subarachnoid space; the finding argues strongly for the belief that the major portion of the cerebrospinal fluid is produced within the cerebral ventricles. But Dandy's (16) later experiments constitute dependable evidence not only that the place of production of cerebrospinal fluid is intraventricular but that the choroid plexuses are the responsible structures. Dandy was able to produce a unilateral internal hydrocephalus by obstructing one foramen of Monro; extirpation of the choroid plexus in such an obstructed lateral cerebral ventricle prevented the development of an internal hydrocephalus. Dandy's experiment furnishes the strongest single substantiation of the hypothesis that the choroid plexuses elaborate the cerebrospinal fluid.

From an entirely different aspect, also, corroborative evidence in favor of the choroidal origin of the cerebrospinal fluid is found in embryological observations of the writer (69). In a study of the development of the cerebrospinal spaces it was shown that the first extraventricular expulsion of the cerebrospinal fluid occurred simultaneously with the first tufting and histological differentiation of the ependymal cells to form the choroid plexuses.

The evidence, then, from histological, pharmacological, pathological and embryological standpoints, surely inclines one to acceptance of the hypothesis that the choroid plexuses of the cerebral ventricles largely elaborate the cerebrospinal fluid. It does not seem justifiable to accept the evidence from any one standpoint as conclusive for many of the observations recorded are corroborative only. Yet the general histological structure of these plexuses, the cytoplasmic inclusions, and the modification of the cell-structure by pharmacological agents offer more than suggestive substantiation of the contention. The pathological studies of cases of internal hydrocephalus, the direct observations of the "sweating" choroid plexus, the embryological relationship between differentiation of choroid plexuses and extraventricular spread of the ventricular fluid, and particularly the experimental investigation of unilateral hydrocephalus, when considered as a whole, present a very strong argument, if not wholly conclusive, in favor of the view that the choroid plexuses are the elaborators of the major portion of the cerebrospinal fluid. It does not seem justifiable to discard, as Becht (1) has done, all of the evidence in favor of this hypothesis as inconclusive. While many of the experimental findings, when viewed as isolated observations, may be explained by other hypotheses, the great mass of data cannot be interpreted on any other hypothesis as satisfactorily. Certain of Becht's specific objections to acceptance of the theory of origin of the liquid from the choroid plexuses have been answered within the last year: Wislocki and Putnam (79) demonstrated by histological methods that in cases of experimental internal hydrocephalus there was absorption of foreign solutions through the ependymal cells lining the ventricles but not through the cells of the choroid plexus. These observations were confirmed and extended by Nafnagas (53), who showed by similar procedures that an increased intraventricular absorption of fluid occurred after the intravenous injection of hypertonic solutions of sodium chloride; in no case was there absorption of the fluid by the cells of the choroid plexus. With this evidence in hand, it seems justifiable to disregard Becht's contention that the cell-changes in the choroid

plexuses, reported by Pettit and Girard and by Meek, may as properly be interpreted as indicative of absorption as of secretory activity.

But even as a working hypothesis, the choroid plexuses must not be considered to be the sole elaborators of the cerebrospinal fluid. Anatomical evidence presented by the writer (68) indicates that the perivascular spaces also pour a certain amount of fluid into the subarachnoid space, where this fluid mixes with the liquid produced in the cerebral ventricles. Such an addition to the cerebrospinal fluid probably accounts for the reported differences between subarachnoid and ventricular fluids on serological and chemical analysis. The ependymal cells lining the cerebral ventricles and the central canal of the spinal cord may also contribute even in the adult a minimal addition to the intraventricular cerebrospinal fluid.

Although a constant elaboration of cerebrospinal fluid by these mechanisms is indicated, it is not known how large a quantity is produced in any given time-interval but it is not unlikely that the majority of computations are by far too large. Most of the estimates in man have been based on the amount of fluid pouring from subarachnoid fistulae (also cases of cerebrospinal rhinorrhea) where the pressure, against which the fluid is produced, is determined solely by the resistance of the abnormal pathway. The same lack of normal conditions renders unreliable the determinations which are based on the rate of flow from experimental cannulae or fistulae. Estimations of the production of fluid based on the absorption of foreign dyes likewise may lead astray. The evidence indicates, however, that there is a constant though not excessive elaboration of the cerebrospinal fluid; the computations of exact quantities thus far given are of but little value.

#### CIRCULATION OF THE CEREBROSPINAL FLUID

The cerebrospinal fluid produced largely by the choroid plexuses is poured directly into the cerebral ventricles which are lined by ectodermal ependymal cells. That portion of the fluid formed in the lateral ventricles flows through the foramina of Monro into the third ventricle and thence by the aqueduct of Sylvius into the fourth ventricle. From the fourth ventricle the fluid passes out into the subarachnoid space; there is no evidence that functional communications between cerebral ventricles and subarachnoid space exist elsewhere than in this region.

The exact mode of escape of the ventricular cerebrospinal fluid from the fourth ventricle into the subarachnoid space must still be considered as slightly uncertain. It is possible that the inferior velum of the cere-

bellar roof in the adult is an intact though functioning membrane, as in the embryo; the existence of a perforation (the foramen of Magendie) in this membrane has been termed an artifact because of the dislocation of structures necessary to demonstrate it macroscopically or because of the shrinkage of tissues in embedding for histological investigation. The greater weight of evidence today inclines to a consideration of the foramen of Magendie as a true anatomical opening in the velum—a break in the ependyma and pia. In support of this conception of a true foramen between fourth ventricle and subarachnoid space may be quoted the observations of Cannieu (8) and of Wilder (78) and especially the developmental studies of Hess (38) and of Blake (6). Blake's conception of the formation of this opening—a shearing-off of the base of a finger-like evagination of the rhombic roof—is rendered more certain by recent morphological studies of this region. The two lateral foramina—those of Luschka—connecting the lateral recesses of the fourth ventricle with the subarachnoid space, seem to have as established a basis for their existence as does the medial. It is through these three foramina—or surely in the region of the inferior tela choroidea if through an intact membrane—that the cerebrospinal fluid, produced in the cerebral ventricles, passes into the subarachnoid space.

From the cisternal dilatation of the subarachnoid space in the region of the medial cerebello-bulbar angle the cerebrospinal fluid slowly seeps downward in the spinal subarachnoid space but passes more rapidly upward about the base of the brain and thence more slowly over the hemispheres, surrounding the whole central nervous system. This movement of fluid is facilitated by impulses transmitted to it by the vascular system; in the spinal region there is also an almost equivalent passage of fluid upward. The subarachnoid space, in which the fluid circulates, is, according to current anatomical descriptions, contained between the arachnoidea and the pia mater. Such a description does not present a proper conception of these fluid-containing channels, for it seems far preferable to consider the subarachnoid space to be intraleptomeningeal. On this basis the arachnoid may be described as the outer continuous membrane, intact and fluid-containing, from the inner surface of which project numerous delicate trabeculae, which merge with the pia mater. The surfaces of the arachnoid membrane and of the trabeculae are covered, as is the inner surface of the dura mater, by flattened, polygonal mesothelial cells. Identical cells also clothe the surface of the brain and spinal cord to form the essential cell-covering

of the pia mater. All structures (blood vessels, nerves, etc.) traversing the subarachnoid space are likewise covered by these mesothelial elements. The cerebrospinal fluid, hence, is contained within a completely cell-lined system of continuous yet partially interrupted spaces in the meshes of the arachnoid trabeculae. These meshes are of various sizes, increasing from very fine reticular spaces over the cerebral hemispheres to more widely calibered channels in the cerebral sulci and about the spinal cord, and reaching their maxima in the cisternal dilatations about the cerebello-bulbar angle. In the wide channels of this subarachnoid meshwork the cerebrospinal fluid is obstructed but little in its circulation, but in the small meshes the flow of the liquid is slowed.

Apart from their established function as efficient fluid-retainers, the cells lining the subarachnoid space are of great interest because of their changing morphology under different physiological conditions. The writer (70) first noted that these mesothelial cells phagocytosed carbon granules introduced into the subarachnoid space and that when phagocytic, the cells increased in size. Essick (24) then showed that the presence of foreign material (laked blood, granules, etc.) caused these cells to become enlarged, vacuolated, phagocytic and finally detached to form free macrophages of the cerebrospinal fluid.

These mesothelial cells likewise have importance in establishing the relations between the subarachnoid and the perivascular spaces, for there is everywhere in the central nervous system a distinct fluid-containing space about each of the perforating blood vessels. The cells of the pia mater turn inward to form an outer wall of such a perivascular channel while the cells of the arachnoid, covering the vessel as it traverses the subarachnoid space, are likewise continued inward to form an inner cuff of this space. Thus each blood vessel penetrating the nervous system is surrounded by a cell-enclosed, peri-adventitial fluid-channel, which communicates directly with the subarachnoid space. The typical leptomeningeal mesothelial cell of this channel has been identified for variable distances from the surface, dependent upon the caliber of the penetrating vessel. The perivascular fluid-channel, when the mesothelial cell-cuff ceases, continues inward to connect directly with perineuronal spaces about the nerve-cells. These ultimate fluid-spaces are potential in character but under certain circumstances they become easily recognizable in microscopic preparations (Mott (52)). While originally termed "lymphatic" in character, these perivascular channels have no connection with the lymphatic system; they represent however an important accessory fluid-system of the cerebrospinal axis, affording direct

pathway, uninterrupted by cell-membranes, between nerve-cell and subarachnoid space.

Thus far mention of the dura mater has been omitted, for it has but slight relationship to the cerebrospinal fluid, the subdural space being anatomically and probably physiologically entirely apart from the subarachnoid. But in one respect the dura mater has importance in the present problem: the areas of penetration of the dense fibrous tissues of the dura by the arachnoid represent points of fusion between the two membranes. The most frequent of these areas of penetration are the arachnoid villi—prolongations of the arachnoid membrane so that the arachnoidal mesothelial cells come to be directly beneath the vascular endothelium of the great dural venous sinuses. Identified in adult man, in infants and in the common laboratory mammals (Weed (66), (67)), these villi are covered by typical arachnoid cells, usually of a single layer but often forming whorls and presenting double-layered coverings. The core of such a villus may be a strand-like network reduplicating the subarachnoid space or a myxomatous ground work simulating the perimedullary mesenchyme. In addition to the true arachnoid villi, which occur in the walls of practically all of the dural sinuses, there are found infrequently prolongations of the arachnoid into the dura in other situations. The arachnoid villi are normal structures; the great enlargement of these in adult life results in the formation of the well-known Pacchionian granulations.

The cerebrospinal fluid, then, circulates everywhere about the central nervous system—in the cerebral ventricles and central canal of the spinal cord and also in the tortuous meshes of the subarachnoid space. These channels are all clothed with a specialized cell, fluid-retaining so that a true circulation of fluid may be maintained. And in the arachnoid villi the circulating fluid comes into close relationship to the great venous sinuses of the dura mater.

#### ABSORPTION OF THE CEREBROSPINAL FLUID

With the evidence indicating a constant production of cerebrospinal fluid and a circulation of the liquid through the cerebral ventricles and throughout the subarachnoid space, it is not surprising that many investigations should have been undertaken to determine the mode of absorption of the fluid. The experiments performed fall naturally into two groups—the physiological observations to determine whether absorption of the fluid is into venous system or lymphatic trunks and the anatomical investigations to ascertain the exact pathways along which the fluid is

absorbed. Because of the great importance of this phase of the subject the evidence will be given in some detail.

Modern anatomical studies of the pathways of absorption of the cerebrospinal fluid were first made by Key and Retzius (43) who injected into the spinal subarachnoid space of a cadaver a gelatine solution colored with Berlin blue. While the pressure used was somewhat excessive (60 mm. Hg.), the anatomical continuity of spinal and cranial subarachnoid spaces was demonstrated, for the whole cranial subarachnoid space was filled with the gelatine-mass. Furthermore, the gelatine was traced into the core of the Pacchionian granulations along the great dural sinuses, and through the cell-membranes directly into these venous sinuses. Many beautiful plates showing this passage of the injection-mass but giving evidence of possible rupture are presented in Key and Retzius' monograph. In addition to this major pathway of absorption, these investigators demonstrated a minor accessory absorption of the fluid into the lymphatic system. Quinke's (56) confirmatory observations, though appearing before the monograph of Key and Retzius, did not antedate their earlier papers on the subject. Quinke injected into the subarachnoid space of living animals a suspension of cinnabar granules and, killing the animals at varying periods thereafter, discovered the granules almost wholly within the basilar and spinal subarachnoid spaces, for the most part held by phagocytic cells. Granules were also found along the venous sinuses in structures which he termed Pacchionian granulations; in the cervical lymph nodes likewise particles of the sulphide were identified.

For several years after these publications, the view of Key and Retzius was accepted as establishing the anatomical pathway of absorption of the cerebrospinal fluid. But gradually with the realization that Pacchionian granulations as such do not exist in infants and in the higher mammals, it was felt that this view was inadequate. For the next two decades practically no work was done upon the subject; then suddenly renewed interest in the problem was made manifest by the publication of several important physiological observations demonstrating a major absorption of the liquid into the venous system and a minor lymphatic drainage.

Reiner and Schnitzler (57) injected saline solutions containing potassium ferrocyanide into the spinal subarachnoid space of living animals and recovered the foreign salt in from 30 to 40 seconds from the blood of the jugular vein. Olive oil injected under similar conditions was identified likewise, though the blood stream was slowed. Reiner and

Schnitzler stated that as Pacchionian granulations do not exist in the animals used, other pathways of absorption must exist. Shortly thereafter Leonard Hill (39) reported that saline solution, colored with methylene blue and introduced into the subarachnoid space, could be traced "straight into the venous sinuses." Within a few minutes (10 to 20) after the injection, the blue was identified in the bladder and stomach; the cervical lymphatics became colored only after an interval of one hour. Following injection of potassium ferrocyanide into the cerebrospinal fluid, Ziegler (81) detected the foreign salt in the posterior facial vein in 10 seconds and only after 30 minutes in the cervical lymphatics. Similarly, Lewandowsky (44) identified sodium ferrocyanide in the urine of animals within 30 minutes after intraspinal introduction.

The experiments of Spina (63), though conducted under abnormally high pressures, likewise add support to the idea of a very rapid major venous absorption and a lesser lymphatic drainage. Cushing's (13) observations on mercury and non-absorbable gases led him to hypothesize a valve-like mechanism for drainage into the venous channel. Both of these observers commented upon the absence of Pacchionian granulations in the higher mammals.

Mott (52) in 1910, from study of the brains of animals subjected to experimental cerebral anemia, suggested a new pathway for the absorption of the cerebrospinal fluid, based on the occurrence of distinct spaces about each nerve-cell, connected through the perivascular channels with the subarachnoid space. Believing that this fluid-system contained cerebrospinal fluid, Mott contended that normally the liquid passes from subarachnoid space into cerebral capillaries. Cathelin (10), without supporting data, assumed that the major absorption of the cerebrospinal fluid was into the lymphatic system; and Goldmann (36), employing subarachnoid injections of trypan blue, likewise tentatively favored this view, though acknowledging the weight of evidence in favor of the major venous drainage.

Dandy and Blackfan (17) in 1913 concluded that the absorption of cerebrospinal fluid was a "diffuse process from the entire subarachnoid space," for with the spinal subarachnoid space isolated from the cranial, they found "a quantitative absorption proportionately as great as from the entire subarachnoid space." The evidence for these statements (18), published in detail a year later, was largely based on the excretion by the kidneys of a readily diffusible dye—phenolsulphonephthalein—after its introduction into the subarachnoid space. A very rapid

absorption into the blood stream occurred under such experimental conditions while the lymphatic drainage was minimal in amount and very tardy. After subarachnoid injections of india ink, Dandy and Blackfan were able to find no anatomical evidence of absorption of the carbon granules—an observation in accord with those of Quineke (56) and of Sicard and Cestan (62).

It was with these contributions as a background that the writer (66), (67), (68) began his anatomical studies of the absorption of the cerebrospinal fluid. Critical examination of the preceding work was convincing in demonstrating a major venous absorption and a minor lymphatic drainage of the liquid, but the reliable data were practically entirely physiological. Thus the observations regarding the rapid venous absorption of readily diffusible substances seemed wholly dependable but the anatomical evidence was satisfactory solely in demonstrating that insoluble particles (cinnabar, carbon) did not leave the subarachnoid space in any great amount. The only complete morphological investigations were those of Key and Retzius; these were unsatisfactory because the injections were performed on dead material, the pressures employed were high (60 mm. Hg.) and the injection-mass was a viscous colloid (gelatine) colored with Berlin blue. It was felt that the experimental approach must be such that a subarachnoid injection of a true, isotonic solution of non-toxic foreign salts, capable of subsequent precipitation *in situ* for histological examination and not diffusely staining cellular material, could be made in the living animal, under pressures not greatly in excess of the normal. With these criteria established, experiments were undertaken with subarachnoid injection of potassium ferrocyanide and iron-ammonium citrate in isotonic solution under pressures but slightly above the normal (130–180 mm. H<sub>2</sub>O). Subsequently the central nervous system, enclosed in meninges, was fixed in an acid medium; precipitation of the foreign salts as Prussian blue permitted adequate histological identification of the pathway taken. This method was found to meet all of the standards of investigation set.

The experiments were carried out over periods of several hours in living anesthetized animals, with introduction of the isotonic foreign solution into the lumbar subarachnoid space. The spinal and basilar portions of the subarachnoid space were rapidly filled with the foreign solution but the cerebral portion of the space was not completely injected until the experiment had been continued for several hours. Histological examination demonstrated that the solution had not pene-

trated any of the cells lining the subarachnoid space; the precipitated granules adhered to the surfaces of the cells but were not within the cytoplasm. The foreign solution was found to have passed directly into the venous sinuses by way of the arachnoid villi into which the precipitated granules could be traced from the cerebral subarachnoid space. These granules, representing the foreign solution, were found within the mesothelial cells covering the villi and the endothelial cells lining the venous sinuses, as well as within the lumen of the venous sinus, thus demonstrating the essential pathway of absorption. In no other place was there evidence of direct passage through a cell-membrane as in the villi. The mechanism of passage of this fluid seemed to be a process of filtration from a point of higher pressure (subarachnoid space) to a point of lower pressure (venous sinus), though the factors of osmosis and diffusion were not excluded. The absorption of true solutions from the cranial subarachnoid space was shown to be a much more efficient and rapid process than was the corresponding absorption from the spinal subarachnoid space. Suspensions of particulate matter were found, in agreement with the observations already recorded, to be retained within the meshes of the subarachnoid space.

In addition to this major venous absorption through arachnoid villi directly into the great dural sinuses, an accessory drainage by way of the lymphatic system was demonstrated. This seemed a much slower, less efficient means of absorption of the fluid, caring for but a small fraction of the total. Such lymphatic absorption was wholly indirect; the fluid reached the true lymphatic vessel only outside of the dura and then by way of perineural spaces.

These anatomical findings, based on a standard of experimentation which approximated the normal, agreed largely with those of Key and Retzius, substituting however for the Pacchionian granulation the normal arachnoid villus. Further observations were made to ascertain the truth of the other anatomical hypotheses ventured for the absorption of cerebrospinal fluid; thus, no structure of a valve-like nature was found in many examinations of serial sections of the great dural sinuses. Mott's theory of absorption by cerebral capillaries was excluded by the failure of the injection-fluid to pass into the perivascular system under conditions approaching the physiological. Dandy and Blackfan's conception of a diffuse absorption by the vessels of the subarachnoid space was likewise found untenable, for in no case were the mesothelial cells covering these vessels penetrated by the foreign solution. The strongest argument in favor of the diffuse process of absorption advanced by

Dandy and Blackfan was that the excretion of phenolsulphonephthalein from the isolated spinal subarachnoid space was proportionately as great as from the whole cranial and spinal subarachnoid space. The technique employed by Dandy and Blackfan involved withdrawal of an equivalent amount of cerebrospinal fluid from the isolated spinal subarachnoid space before injection of 1 cc. of the phenolsulphonephthalein solution. The writer (67) believed that this substitution could not be made in this isolated space without increase in the spinal subarachnoid pressure and escape of the foreign dye into the epidural tissues. By reversing the experiment he was able to show that absorption of phenolsulphonephthalein when introduced into the cisterna magna was as rapid when the spinal subarachnoid space was excluded as with the whole cranial and spinal subarachnoid space functioning. The cranial portion of the nervous system seems, therefore, to contain the efficient mechanisms for the absorption of cerebrospinal fluid.

Since the publication of this work in 1914 there have been other observations regarding the absorption of the cerebrospinal fluid; all accord with this idea of a major venous absorption of the fluid. Thus Frazier and Peet's (33) experiments with methylene blue, isamine blue, trypan red, trypan blue and phenolsulphonephthalein demonstrated the greater importance of the rapid venous absorption and the lesser of the slow lymphatic drainage. And Dixon and Halliburton (21), in the course of their studies of the cerebrospinal fluid, confirmed this general conception of the process, finding no escape of particulate matter from the subarachnoid space but a free and rapid absorption of true solutions. Between the two types there occurred a much slower absorption of colloidal solutions than of true solutions, the larger molecules being absorbed more slowly than the smaller. They concluded, as did also Halliburton (37) that "the fluid probably reached the venous sinuses by way of the microscopic arachnoid villi." And recently the writer, in work as yet unpublished, has repeated certain phases of his original investigation of the pathways of absorption, with continuous observations of the pressures of the cerebrospinal fluid and of the intracranial blood vessels. The results confirm in every way the conception of the mechanism here presented.

Thus it seems fair to assume that the absorption of the cerebrospinal fluid is a twofold process, being chiefly a rapid drainage into the great dural sinuses, and in small part a slow indirect escape into the true lymphatic vessels.

## THE PRESSURE OF THE CEREBROSPINAL FLUID

Practically all of the methods of recording the pressure of the cerebrospinal fluid deal with connection of the subarachnoid space to an open or membrane manometer. It is very difficult with any of the procedures to avoid the loss of a few drops of fluid, but by immediate replacement of this liquid and by the use of manometers filled to the estimated level of the fluid, the pressures obtained by these simple methods may be accepted as accurate.

Very few, if any, of the early records of the pressure of the cerebrospinal fluid were sufficiently controlled to permit direct comparison with the data obtained by recent workers. As examples of the variation in the pressures recorded by the earlier investigators, the following determinations may be given: Key and Retzius (43), 162 to 216 mm.  $H_2O$  in inspiration and 216 to 270 mm.  $H_2O$  in expiration, in etherized dogs; Bergmann (4, 5), 80 mm.  $H_2O$  in his first observations and in his second series, 120 to 160 mm. of salt solution in narcotized dogs; Falkenheim and Naunyn (26), 100 to 150 mm.  $H_2O$  in curarized dogs; and Leyden (45), 80 to 150 mm. and 100 to 120 mm.  $H_2O$  in dogs under morphia. Leonard Hill (39) believed, however, that the intracranial tension might vary from zero to 50 mm. Hg. and that the variations reported by the earlier observers were but expressions of this characteristic of the intracranial pressure.<sup>1</sup> Hill's idea of variability in the normal pressure of the cerebrospinal fluid seemed supported by the observations of Dixon and Halliburton (20), who reported 40 to 70 mm. of salt solution as a rough average of the normal pressures obtained in the dog under morphine-urethane anesthesia. And in a single detailed protocol presented, the pressures at 5-minute intervals ranged as follows: 95, 25, 30, 35, 55, 25, 80, 65, 65, 65, 75, 70, 60, 55, 50, 80, 90 mm. of 10 per cent citrate solution.

The most recent work, particularly by American investigators, has given much better knowledge of the range and variability of the normal pressure of the cerebrospinal fluid. Weed and McKibben (75) reported an initial average of 119 mm. of Ringer's solution in cats anesthetized by intratracheal ether, and an extreme constancy of the pressure of the cerebrospinal fluid under such experimental conditions. Becht (1) found considerable fluctuation in the pressure of the fluid in etherized dogs but of lesser extent than did Dixon and Halliburton; his average

<sup>1</sup> Within the limits of the physiological phenomena investigated, intracranial pressure may be considered to be identical with that of the cerebrospinal fluid.

pressure of 112 mm. (sodium chloride solution of specific gravity of 1.088) was derived from 39 dogs under intratracheal ether. Foley and Putnam (31) presented a slightly higher average of 127 mm. for the normal reading in animals under ether while the average of 100 animals under various anesthetics was 133 mm. of cerebrospinal fluid; no comment upon the extent of normal fluctuations of the fluid pressure was made. And recently Weed and Hughson (72) have reported an average pressure of 119 mm. of Ringer's solution for 77 cats under ether by Woulfe bottle; the fluctuations in the pressure under the experimental conditions were very slight in extent (11 mm. in an animal under observation for 2 hours).

The question of greatest physiological interest in this phase of the general problem is that of the maintenance of this pressure of the cerebrospinal fluid. All of the conceptions of the mechanism for the maintenance of this pressure are based primarily upon the rigid character of the bony coverings of the nervous system. This idea that the cerebrospinal axis is situated within a "closed box," to which the physical laws of such a system apply, was first advanced in 1783 by Alexander Monro (51). Monro believed that the substance of the brain, like that of other solids of the body, is nearly incompressible and is "enclosed in a case of bone," assuring therefore the constancy of the intracranial blood content. The development of this hypothesis by Kellie (42) in 1824 led to wide acceptance, and the Monro-Kellie doctrine with but few alterations has served as the basis upon which the physiology of the intracranial contents has been interpreted. The doctrine was modified by the introduction of the cerebrospinal fluid into the consideration by Burrows (7) under the influence of Magendie's epochal studies; the hypothesis was then formulated by Burrows as follows (p. 32): "the whole contents of the cranium, the brain, the blood and this serum (cerebrospinal fluid) together, must be at all times nearly a constant quantity."

Many physiologists have subjected this Monro-Kellie doctrine to experimental proof not only in dead but in living animals; while divergent conclusions have been arrived at, the general consensus of opinion expressed in the literature of the last century has been in favor of the hypothesis. And in the hands of recent workers a similar unanimity of expression holds though occasional investigators express doubt as to the accuracy of the premise. Lately, Weed and Hughson (73) have experimentally demonstrated the essential truth of the doctrine that (p. 99) "the bony coverings of the central nervous system constitute

within tested physiological limits, inelastic and rigid containers; the ordinary physical laws of a 'closed box' may therefore be applied to the cranium." And with appreciation of the variability in volume of the constituents, the hypothesis may be stated, as was done by Weed and McKibben (76, p. 553): "the cranial cavity is relatively fixed in volume and is completely filled by brain, cerebrospinal fluid and blood; variations in any one of the three elements may occur, compensation being afforded by alteration in the volume of one or both of the remaining elements."

With the cranium and vertebral column serving as rigid containers, the relation of the intracranial vascular pressures to the pressure of the cerebrospinal fluid requires immediate consideration. Current physiological conceptions of these intracranial mechanisms really date from the work of Leonard Hill (39) who advanced the idea that intracranial pressure is (p. 71) "the same as cerebral venous pressure, and varies in the same direction absolutely as general venous pressure, and proportionately as general arterial pressure." Hill's technical procedures consisted in determining cerebral venous pressure in the torcular Herophili and cerebrospinal fluid pressure in the occipital region.

The emphasis placed by Hill upon this equality of pressure dominated physiological opinion for over fifteen years; it was not until the publication of Dixon and Halliburton (20) in 1914 that contradictory evidence was presented. Using experimental procedures essentially similar to those of Hill, Dixon and Halliburton demonstrated that the cerebrospinal fluid pressure is not identical with that of the dural venous sinus, and stated that the fluid pressure is (p. 153) "influenced passively to a small extent by changes in the arterial and venous pressures but such alterations are insignificant compared with the independent changes in pressure which occur as the result of secretory activity." These investigators also showed that increase in the cerebrospinal fluid pressure produced a passive increase in the cerebral venous pressure but not of the same extent; conversely, alteration of the cerebral venous tension caused similar though not identical alteration in the pressure of the cerebrospinal fluid. Under normal conditions the arterial pressure was found by Dixon and Halliburton to be higher than the intracranial venous pressure which in turn was always higher than that of the cerebrospinal fluid.

Becht (1), employing somewhat similar methods, came to conclusions in some respects at variance with those of Dixon and Halliburton though confirming the general contention of inequality of cerebrospinal fluid

pressure and cerebral venous pressure. Becht stated that the cerebrospinal fluid pressure was dependent upon both intracranial arterial and venous pressures, though not identical with either. The data obtained indicated that either the cerebral venous (torcular) or cerebrospinal fluid pressure might be the higher but that usually the former exceeded the latter. Passive changes in the torcular pressure were found to affect the pressure of the cerebrospinal fluid, in the same direction but not to the same extent; but within fairly wide limits changes of the cerebrospinal fluid pressure were not believed to alter the pressure of the torcular.

The observations of these recent workers were all carried out with fairly similar technical procedures, particularly for the determination of venous pressure in the torcular Herophili. The very wide divergences in these normal pressures (13 to 601 mm. in Becht's series) indicate that the method is subject to many experimental defects. Weed and Hughson (74), with these technical disadvantages in mind, devised a simple method for recording intracranial venous pressure in the superior sagittal sinus as it empties into the torcular. The procedure possessed the very distinct advantage of permitting direct observations of the effect of the manipulative procedure upon the pressure of the cerebrospinal fluid, thus affording control of artificial increases in the pressure of the cerebrospinal fluid due to venous obstruction in the cranium. With such technical controls, Weed and Hughson were able to show that in practically every case the pressure of the cerebrospinal fluid was considerably above (5-50 mm.) that of the sagittal sinus. They also presented data which indicated, in accordance with the findings of Dixon and Halliburton and of Becht, that alteration in intracranial venous pressure effected alterations in the pressure of the cerebrospinal fluid in the same direction but of lesser magnitude. Conversely it was shown, in agreement with Dixon and Halliburton, that within the physiological limits tested, alteration in the pressure of the cerebrospinal fluid caused changes of lesser extent but of the same direction in the sagittal venous pressure.

This conception, advanced by Weed and Hughson, that the pressure of the cerebrospinal fluid is practically always above that of the cerebral veins, alone affords basis of explanation for Wegfarth's experiments. Wegfarth (77) demonstrated that an experimental communication between subarachnoid space and superior sagittal sinus remained patent, without hemorrhage into the meningeal cavities, for at least 4 days. Removal of cerebrospinal fluid in these animals, however, resulted in

immediate intrameningeal hemorrhage. Such an observation, free from the errors of recording instruments, can be explained only on the basis that normally the pressure of the fluid is above that in the sinus or that the former is constantly being reduced toward the latter.

Analysis of the reliable data concerning these normal relationships seems convincing in demonstrating that the pressure of the cerebrospinal fluid practically always exceeds that of the superior sagittal sinus. In no sense may the two pressures be regarded as identical, for such an identity of pressures is found only in animals in which the technical procedures have resulted in the production of direct communications between sinus and meningeal spaces. In the normal animal intracranial arterial pressure is a factor of importance in the maintenance of the pressure of the cerebrospinal fluid, though slight or slowly effected changes in this arterial tension have but little influence upon the fluid pressure. Thus while dependent upon both intracranial arterial and venous pressures and while influenced passively and in the same direction by both, the pressure of the cerebrospinal fluid may be considered to be relatively independent of both in that normally it maintains an individual, fairly constant level far below that of the intracranial arteries and somewhat above that of the intracranial veins.

It becomes desirable, then, to ascertain the factors which determine the level of the cerebrospinal fluid pressure, but in this inquiry there are practically no data available and the problem becomes speculative. Yet it is instructive to think of the cerebrospinal fluid as being largely elaborated by the cells of the choroid plexuses where the pressure in the blood capillaries is estimated at from 40 to 60 mm. Hg. On the outer side of these cells is the cerebrospinal fluid with its pressure of 110 to 130 mm. of Ringer's solution. After circulating through the cerebral ventricles and subarachnoid space this fluid is largely returned into the venous sinuses of the dura where the pressure (as determined in the superior sagittal sinus) is below that of the cerebrospinal fluid (as determined in the cisterna magna). On such a basis the normal mechanism for the absorption of this characteristic body liquid may well be a simple process of filtration though the factors of osmosis and diffusion are not excluded in the passage of the fluid through the cell-membrane of the arachnoid villus. No determinations of the pressure of the cerebrospinal fluid in the arachnoid villus have been made but it is unlikely that this pressure is markedly different from that of the cisterna magna. Obstruction to any part of the pathway of the fluid results in raising intraventricular tension (cf. Nafagás (53)) thus demonstrating that

cerebrospinal fluid can be produced against higher pressures than normally exist in the cerebral ventricles. The normal pressure therefore may be largely determined by the balance between the constant new production within the cerebral ventricles and the absorption into the dural sinuses: this pressure of the cerebrospinal fluid becomes dependent also upon intracranial arterial and venous pressures, not only because of the relation of these latter pressures to the production and absorption of the fluid, but because of the constancy of volume of the intracranial contents.

#### MODIFICATION OF THE PRESSURE OF THE CEREBROSPINAL FLUID

During the past ten years a number of investigators has studied the alterations in pressure of the cerebrospinal fluid effected by the introduction of various substances into the blood stream or alimentary canal. The subject-matter naturally differentiates itself from the purely mechanical modifications effected by pressure-changes in the cerebral blood vessels. The general findings in this latter group of experiments have been presented in the foregoing section of this review; the pressure-changes effected in the cerebrospinal fluid by solutions of various concentrations and by certain pharmacological agents and tissue extracts will be discussed here.

*Solutions of various concentrations.* In 1919 Weed and McKibben (75) reported that the pressure of the cerebrospinal fluid could be markedly altered by the intravenous injection of solutions of various concentrations. It was shown that such administration of strongly hypertonic solutions lowered the pressure of this liquid to an extreme degree, frequently producing negative values; with hypotonic solutions (distilled water) a prolonged rise in the pressure of the fluid was obtained. Ringer's solution in large doses produced a temporary increase in the pressure of the cerebrospinal fluid, followed quickly by a return to normal levels. Accompanying these changes in fluid pressure, Weed and McKibben (76) found marked alterations in the volume of the brain, the hypertonic solution producing a small shrunken brain while the hypotonic solution caused an outspoken swelling of the brain-substance. The experimental changes in brain volume were particularly pronounced in animals in which the cranial cavity had been opened by trephining.

These physiological findings have since been abundantly confirmed and clinical applications of the phenomena have been developed. Cushing and Foley (15) demonstrated that similar alterations in the

pressure of the cerebrospinal fluid could be brought about by the ingestion of hypertonic and hypotonic solutions. Subsequently Foley and Putnam (31), after verifying the original conclusions, studied similar changes in the pressure of the cerebrospinal fluid which were effected by the intra-intestinal administration of these solutions of various concentrations. And Sachs and Malone (60) reported observations upon the decrease of brain volume caused by the intravenous injection of strongly hypertonic solutions. In addition to these papers, there has appeared a number of reports of clinical application of this experimental modification of fluid pressure or brain bulk—notably those of Cushing and Foley (15), Sachs and Belcher (59), Ebaugh and Stevenson (22), Foley (30) and Hughson (40).

Recently Weed and Hughson (72), (74) have extended the original observations of Weed and McKibben (75); in addition to confirming the initial work in detail, they have presented data showing the general systemic and intracranial vascular alterations effected by these agents. These observations were made over periods of from 2 to 7 hours, under adequate experimental conditions in which the pressures recorded (cerebrospinal fluid, carotid artery, superior sagittal sinus, superficial brachial vein) remained surprisingly constant. The intravenous injection of a large quantity of Ringer's solution was shown to cause a temporary rise in the pressure of the cerebrospinal fluid with increases in both sagittal and brachial venous pressures, the former being the greater. At the end of the injection-period all of these pressures tended to return to their previous levels, normal pressures customarily being attained within 30 minutes. After the intravenous injection of distilled water in similar amount, a prolonged rise in the pressure of the cerebrospinal fluid occurred, accompanied by alterations in both sagittal and brachial pressures. Both of these venous pressures increased during the period of injection and for a few minutes thereafter, the sagittal outstripping the brachial; within 30 minutes these pressures were usually returned to their pre-injection levels, though the pressure of the cerebrospinal fluid was still elevated. With the intravenous injection of strongly hypertonic solutions, the pressure-alterations were most striking, the cerebrospinal fluid, after a frequently occurring rise during the interval of injection, dropping markedly and often exhibiting extreme negative values. The pressure in the superficial brachial vein rose during the period of the hypertonic injection and then rapidly resumed its pre-injection level or a new level slightly below. More significant were the alterations in sagittal venous pressure: here the reaction during the

period of injection depended largely on the reaction of the pressure of the cerebrospinal fluid, but following this the sagittal pressure was always lowered to a greater extent than was the brachial venous pressure.

These experiments afforded a unique opportunity for study of the mechanisms which normally control the pressure of the cerebrospinal fluid. Analysis of the data demonstrated that alterations in the pressure of the cerebrospinal fluid could be effected and maintained independently of change in the intracranial and systemic vascular pressures. The most striking similarities in reactions were those between the pressure of the cerebrospinal fluid and that of the brachial vein and sagittal sinus; after the injection of Ringer's solution or of distilled water they exhibited somewhat the same alterations, differing not only in magnitude but in duration. After the intravenous injection of hypertonic solutions, however, the relationships of the pressures were markedly altered, with the pressure of the cerebrospinal fluid profoundly lowered, the sagittal venous considerably and the brachial venous but little if at all. Both brachial and sagittal venous pressures were found to be lower than the pressure of the cerebrospinal fluid in the control-periods; this relationship held after the injection of isotonic and hypotonic solutions but was reversed when hypertonic solutions were given. Arterial changes, when slowly brought about, caused little if any alteration in the pressure of the cerebrospinal fluid, but when abrupt, their influence was marked.

The changes in the pressure of the cerebrospinal fluid, effected by the intravenous injection of solutions of various concentrations, must in the final analysis find their explanation in the alteration of the osmotic pressure of the circulating blood. The injection of a large volume of an isotonic solution was followed by a short-enduring rise of the cerebrospinal fluid pressure which subsided in approximately the same time-interval required for the pressure-changes effected by the hypertonic and hypotonic solutions to reach their maxima. The usual time for maximal reaction of the pressure of the cerebrospinal fluid was noted to be from 25 to 35 minutes after the end of the intravenous injection; in this interval the organism was attempting to compensate for alteration in the volume and salt-content of the blood. With the isotonic solutions, the compensation was one for increased volume of fluid only; this compensation, if judged by the time of return of the cerebrospinal fluid pressure to normal, was rapidly achieved. When, however, not only the volume of the circulating blood was increased but its salt-content relatively diminished as by the intravenous injection of distilled

water, two processes of adjustment proceeded. The blood tended to reestablish its normal salt-content by passage of water into the tissues and possibly into some of the body-fluids, and by attraction of salts from these places; and it also tended to compensate further by alteration of the vascular bed. The increase in the pressure of the cerebrospinal fluid and in the brain volume may be taken to be a rough index of the passage of fluid from blood vessel to tissue; the return of the vascular pressures to normal levels while the pressure of the cerebrospinal fluid remained high, indicated the completion of certain of the phases of readjustment. In the readjustments effected by the organism to the injection of hypertonic solutions, there were somewhat similar phases, yet differing because the great increase of fluid-volume in the circulating blood was not immediate but was due to the attraction of water from the body-tissues and possibly from the body-fluids—a phenomenon shown by the decrease in brain volume and by the reduction of the pressure of the cerebrospinal fluid.

Such an explanation of the phenomena reported leads one naturally to a consideration of the rôle played by the osmotic pressure of the blood in the normal process of absorption of the cerebrospinal fluid. And intimately connected with such a problem is that of the volume of the brain in its relation to the intracranial pressure. At the present time there are available no data which will permit of exact statement; it must be realized that the osmotic changes in the blood, effected by such relatively large injections of solutions of various concentrations, are probably beyond the ordinary physiological limits of change. But if one may judge merely by the anatomical and physiological evidence afforded by subarachnoid injection of isotonic foreign solutions, osmosis and diffusion play subordinate rôles in the normal process of absorption of the cerebrospinal fluid into the blood stream.

*Pharmacological agents and tissue extracts.* Most of the work done on this subject has been actuated by the tenet that alteration in the pressure of the cerebrospinal fluid, without significant change in intracranial vascular pressures, affords a more reliable means of determining the effect of these various agents upon the rate of production of the fluid than the outflow method. While logically this subject should perhaps be discussed under the heading of the modification of the rate of elaboration of the liquid, it may properly be treated here as an experimental alteration of the fluid pressure.

It may be stated at the outset that there is no unanimity of opinion regarding the effect of either pharmacological agents or tissue extracts

upon the pressure of the cerebrospinal fluid, without significant alteration in intracranial arterial or venous pressures. Dixon and Halliburton (20) found that extracts of the choroid plexus, chloroform, ether, urethane, carbon dioxide, amyl nitrite, pilocarpine and other drugs caused a "secretory rise" in cerebrospinal fluid pressure which was independent of the intracranial vascular alterations. Becht (1) has investigated the subject from the same angle, using methods somewhat similar, and has stated that (p. 124) "all the changes in the fluid pressure and in the fluid outflow which have been offered as proof of the secretory mechanism of formation of the cerebrospinal fluids can be traced to alterations in venous and arterial pressures in the skull." Similar conclusions have been reached by Becht and Matill (3) and by Becht and Gunnar (2).

With this conflicting evidence it is of course impossible to do other than reserve opinion in the matter. But certain aspects of the controversy may be commented upon. Dixon and Halliburton and Becht determined cerebral venous pressures in the torcular Herophili—a method which because of the wide divergences in normal pressures reported does not seem adequate though qualitative changes in the pressures are probably fairly accurately shown. While the simple manometric method has been modified by Becht and Gunnar (2), it still has certain limitations in determining a true change in rate of production of cerebrospinal fluid. Of these, the fact that the normal channels of absorption are intact and functioning is the most obvious though Becht has minimized the weight of this objection. There is however a much more formidable disadvantage in that the manometric method cannot take into account the experimental alteration of the volume of the brain. The pressure-changes in the cerebrospinal fluid, effected by the intravenous injection of distilled water, appear to fulfil, after the period of acute vascular change, all of the conditions necessary for the determination that the injection has caused an increased production of fluid—a markedly elevated cerebrospinal fluid pressure with intracranial vascular pressures at the pre-injection levels. The outspoken increase in brain volume under such conditions, however, may well be the sole explanation of the phenomenon; until the experimental variations in brain volume are more fully understood, the evidence obtained by the manometric method should be accepted only with reservations.

## MODIFICATION OF RATE OF OUTFLOW OF CEREBROSPINAL FLUID

That certain pharmacological substances may modify the rate of flow of cerebrospinal fluid from a cannula introduced into the subarachnoid space was first determined by Cappelletti (9) in 1900. Employing Cavazzani's (11) method of making a fistula into the cistern region, Cappelletti showed that ether given by intratracheal tube in a curarized dog increased the rate of flow of the fluid from 0.15 to 0.35 grams per 15-minute interval to 4.72 grams for the same interval. A second, a third and a fourth administration gave momentary increases but not of the same extent as the initial. Similar positive results were obtained with pilocarpine, but the increases though obvious were not marked. Very slight augmentation of the rate of outflow was also obtained with amyl nitrite, while atropine and hyoseyamine caused a decrease and on repetition a cessation of the outflow.

Pettit and Girard (55) immediately confirmed and extended these experimental findings of Cappelletti, including in their studies histological examination of the choroid plexuses. And Meek's (49) observations were likewise entirely confirmatory. The scope of the investigation was widened in 1913 by Dixon and Halliburton (19) who studied the effect of a large number of substances upon the rate of outflow of cerebrospinal fluid from an occipito-atlantoid cannula. They were able to classify the substances into four groups according to their effect on this rate of outflow, placing the volatile anesthetics, alcohol, carbon dioxide and extracts of choroid plexus and of brain in the group which caused marked increase in secretion. Slight increases in the outflow were found to be caused by large injections of water or of normal saline, cholesterol, kephalin, atropine, pilocarpine and amyl nitrite. In the large third group of substances which caused no increase or a diminution of secretion were included extracts of the pituitary, of mussel, of pineal and of pia mater, glucose, urea, lecithin, etc., while in the last group where the effect was possibly masked by vascular or respiratory changes, were muscarin, pilocarpine, adrenalin, etc.

Shortly thereafter Dandy and Blackfan (18), obtaining cerebrospinal fluid by introduction of a special cannula through the atlas, found marked accelerations of the rate of output of cerebrospinal fluid following administration of ether and slight augmentations after pilocarpine. With amyl nitrite and extracts of choroid plexus and of posterior lobe of the hypophysis, no change in the rate of output of the fluid was observed.

Realizing the limitations of this technique in that the normal channels of absorption were intact and that the intracranial pressure was reduced to the resistance of the needle, Weed and Cushing (71) in 1915 catheterized the third ventricle and studied the outflow from the catheter whose resistance was established at approximately normal pressure of the fluid. In addition, cerebrospinal fluid was obtained by callosal and occipito-atlantoid punctures with needles of similarly standardized resistances. Under these circumstances the intravenous injection of extract of posterior lobe of the hypophysis was found to increase the outflow of cerebrospinal fluid. This finding was explained by Dixon and Halliburton (21) on the basis that the hypophysial extract had caused a contraction of the bronchioles and consequent asphyxia. Dixon and Halliburton used an intermittent blast for their artificial respiration while Weed and Cushing employed intratracheal insufflation: it seems questionable whether this explanation of the finding will suffice.

At this time also, Frazier and Peet (34) reported that brain-extract increased the secretion of the cerebrospinal fluid as determined by outflow and that thyroid extract decreased it, independently of any vascular changes.

The later studies of the effect of these substances upon the rate of production of cerebrospinal fluid have been made by the manometric method and have been discussed in the preceding section of this review. The limitations of the outflow method were realized by Weed and Cushing (71) in 1915; their modifications introduced control for some of the sources of error but were incomplete. As Becht (1) has pointed out, practically all of this work is of no scientific value because of failures to record simultaneously the intracranial arterial and venous pressures. Using this standard but employing the manometric method, Becht and Matill (3) have concluded that there is no indisputable evidence that the tissue extracts tested have a specific action on the cerebrospinal fluid. And recently Becht and Gunnar (2) reported that adrenalin, pituitrin, pilocarpine and atropine did not increase the production of cerebrospinal fluid, as determined by manometer readings. It is true that the method of recording the rate of outflow of cerebrospinal fluid from a cistern cannula, even with careful determinations of intracranial vascular pressures, yields unreliable data, but in many ways, also, the manometric method fails. Both of these methods, which at the present time are the only technical approaches to the problem, are of questionable value because they both fail to take account of the experimental variation in brain-bulk. When a method which will permit of actual

determination of this variable brain bulk, with observations also of cerebrospinal fluid pressure and with a record of intracranial vascular pressures, is devised, data of conclusive value will be obtained. And yet one cannot but lay stress upon the changes in the choroidal epithelium recorded by Pettit and Girard (55) after the injection of pilocarpine. Likewise, as first reported by Cappelletti (9) and since noted by many workers, the rapidly decreasing responses to ether and pilocarpine suggest strongly that the accelerations of flow of cerebrospinal fluid under these conditions were not due solely to vascular alteration, for such ready fatigability would not seem to be associated with a vasomotor reaction.

In this connection it is interesting to speculate upon the possibility of modification of the rate of elaboration of the cerebrospinal fluid, after the intravenous injection of solutions of various concentrations. There is as yet no evidence of value in this regard, though Foley and Putnam presented data which suggested that after the injection of a strongly hypertonic solution, a new ratio between the rate of production and absorption of the cerebrospinal fluid became established. But the final elucidation of this phase of the problem will require additional work before definite conceptions are acquired.

#### RELATIONSHIP OF CEREBROSPINAL FLUID TO NERVOUS SYSTEM

Many phases of the relationship existing between the central nervous system and the cerebrospinal fluid are of utmost significance in the present discussion. Filling the cerebral ventricles and central canal of the spinal cord, the fluid also completely surrounds the cerebrospinal axis in the subarachnoid space. This double relationship has prompted many observers to look upon the cerebrospinal fluid as constituting a fluid-cushion for the central nervous system within the closed system of cranium and vertebral column. It has also prompted other workers to liken the cerebrospinal fluid to the lymph of the nervous system—a conception which in the light of present knowledge of the lymphatic system is untenable.

Halliburton (37), in a recent lecture, declared that the cerebrospinal fluid serves as the lymph of the brain, though clearly differentiating it from the true lymph of the lymphatic vessels. It seems likely that such a designation, even when correctly qualified as Halliburton has stated it, is apt to introduce error. All modern investigators of the lymphatic system are agreed that true lymphatic vessels do not exist within the dura mater; the older descriptions of such lymphatic vessels were actu-

ally descriptions of intradural tissue-channels, subpial tissue-channels, or arachnoidal cell-columns within the dura mater. As Sabin (58) has pointed out, our knowledge of the lymphatic system has advanced so that it becomes now necessary to restrict the term "lymph" to the fluid contained within true lymphatic vessels and not to use it to designate any body-fluid.

But in one respect the cerebrospinal fluid does function as an accessory fluid to the central nervous system. In foregoing sections the drainage of the fluid contained within the perivascular channels toward the subarachnoid space has been commented upon; this fluid really becomes added to the ventricular cerebrospinal fluid in the subarachnoid space. In that sense, then, these perivascular spaces represent accessory drainage channels, uninterrupted by cell-membranes and capable of carrying toward the subarachnoid space the waste products of nerve-cell activity. Lacking a true lymphatic system, the nervous tissue apparently makes use of these perivascular channels as pathways for fluid elimination.

The ultimate connection of these perivascular channels with potential spaces about each nerve-cell indicate the close relationship between the cerebrospinal fluid and the nervous system. And in addition to these rather obvious fluid spaces about the nerve-cells, there is evidence indicating that this fluid-system is intimately connected with the general tissue-channels through the ground-substance of the brain. The general direction of flow of this fluid under normal conditions seems to be toward the subarachnoid space.

But under certain conditions this direction of flow may be reversed so that the cerebrospinal fluid passes from subarachnoid space to nerve-cell. The first of these conditions is that of cerebral anemia in which, as Mott (52) showed by histological study, all of the perivascular, pericapillary and perineuronal spaces are dilated. The author (68) made use of this phenomenon as a means of injecting this perivascular system from the subarachnoid space. The second of these conditions under which the perivascular flow is toward nerve-cell, is brought about by the intravenous injection of strongly hypertonic solutions. This phenomenon was first noted by Weed and McKibben (76) who supplied a foreign solution of sodium ferrocyanide and iron-ammonium citrate to the subarachnoid space at the time when the cerebrospinal fluid pressure was approaching zero, following the intravenous injection of a strongly hypertonic solution. This foreign solution was subsequently found (p. 536) "to have passed from the subarachnoid space along the

perivasculars into the substance of the nervous system, reaching the interfibrous spaces in the white matter and the pericellular spaces in the gray." These observations were interpreted as indicating that, under the influence of the intravenous injection of the strongly hypertonic solution, the dislocation of a considerable quantity of cerebrospinal fluid into the nervous system occurred.

Foley (29) has subsequently carried out experiments quite similar to those reported by Weed and McKibben, using the same foreign salts for subarachnoid introduction and intravenous injections of strongly hypertonic solutions. In addition to the findings already detailed, Foley obtained evidence of a retrograde absorption not only by ependyma but by choroid plexuses. The absorption by the ependyma is amply verified by the work of Wislocki and Putnam (79) and Nañagas (53), but these latter workers, using careful histological control, have been unable to obtain evidence of absorption of the foreign salts by the choroid plexuses.

And in work as yet unpublished the writer has repeated many of his earlier experiments done with McKibben, with findings confirmatory in every regard. The intracranial vascular and the cerebrospinal fluid pressures have been determined both before and throughout the period of subarachnoid introduction of the foreign solution, so that definite physiological control is afforded. The results indicate that with the increase of osmotic pressure of the blood, due to the intravenous injection of hypertonic solutions, the cerebrospinal fluid is aspirated into the shrinking nervous system, chiefly along the perivascular channels but also through the ependymal lining of the ventricles. Along these channels, under this extraordinary osmotic pull, actual absorption of the fluid into the vessels of the nervous tissue takes place. The findings suggest a reversal, following the injection of the hypertonic solution, of the normal processes; the osmotic pressure of the blood stream, under these conditions, seems to be a determining factor in the absorption of the cerebrospinal fluid. Interpretation of certain of the experimental observations makes it seem likely that diffusion also plays a part in the process.

#### RÉSUMÉ

The limitations of this review have made it impossible to more than rather briefly discuss a few of the many problems connected with the cerebrospinal fluid. Many equally absorbing phases have been untouched for one or another reason but an attempt has been made to

indicate the type of evidence which has furnished the working hypotheses and to point out the limitations of the procedures on which so many conclusions have been based.

Our present knowledge of the processes of the cerebrospinal fluid in many respects is inadequate. The conception that this characteristic body-fluid is largely produced by the intraventricular choroid plexuses is based not on any single conclusive piece of evidence but on a mass of suggestive data; when considered from all standpoints, however, the hypothesis seems today well established. The current ideas regarding the circulation of the fluid through cerebral ventricles and sub-arachnoid space are founded largely on exact anatomical evidence, particularly in regard to the structure of the meninges and the use of these intrameningeal channels as fluid-pathways. And likewise, there are firm and reliable data of an anatomical and physiological nature supporting the contention that the cerebrospinal fluid is absorbed largely into the venous system and to a lesser extent into the lymphatic channels. It is possible now to discard the hypothesis of equality between the cerebrospinal fluid pressure and that of the cerebral veins, and to regard the cerebrospinal fluid as being maintained at an individual, relatively independent pressure at fairly constant levels above that of the sagittal venous sinus. The conceptions of pressure-changes effected by the intravenous injection of solutions of various concentrations are substantiated by dependable observations, but it does not seem as yet justifiable to accept, without further control, the data furnished in regard to similar changes brought about by administration of pharmacological agents and tissue extracts. And the same cautions may be urged in regard to the acceptance of conclusions based on the effects of various agents upon the rate of outflow of the fluid.

Yet these problems are but few of the many fascinating subjects of investigation in this field. The interesting questions of the chemical composition of the fluid have not been discussed: is the cerebrospinal fluid a true secretion, a transudate, or a modified dialysate? Likewise, the long-debated problems of the passage of foreign salts, of drugs, etc., from blood stream into the fluid must be left for future review, though with possibly a note of suggestion that these investigations be carried out with control of the cerebrospinal fluid pressure. And so may the many other partially answered questions centering about this fluid be enumerated.

But in this field of research the work of the next few years will solve certain problems; yet the solution of these will but expose wider fields

for examination. Here, as in countless other investigations, the study of structure must proceed hand in hand with the study of function, for many of the erroneous conceptions introduced into the literature of the cerebrospinal fluid have been due to failure to give regard to one or other of these basic factors. Future investigations will be the more profitable if the studies be largely along the lines of physiological-anatomical control.

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## MILK SECRETION AS RELATED TO DIET<sup>1</sup>

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The following will serve as a rough outline of the various kinds of experiments which have been carried out to throw light on the physiology of milk secretion as related to diet.

1. Experiments on the chemistry of the various food materials and of milk. A review of the chemistry of milk was published by Raudnitz in 1903 (97).

2. Experiments in which the quantity of particular constituents of the food was varied while the composition and yield of the milk were studied.

3. Experiments in which the income and outgo of various nutritional elements and compounds were studied by determining their quantity simultaneously in the food and in the milk and excreta. Such experiments have been used to throw light on a great number of problems; as, for instance, on the digestibility of various compounds, and on the quantities of these required to supply a given milk production, on the manner in which a lactating animal responds to a shortage of some particular compound in the diet, and on such questions as whether milk fat can come from the carbohydrate or protein of the diet.

4. Experiments on the chemistry of the blood of milking animals. For some time past efforts have been made to determine in this way in what chemical form the immediate precursors of the various constituents of milk are carried in the blood. More recently, the chemistry of the precursors of milk having been to some extent determined, efforts have been made to discover how the concentration of these in the blood is regulated, and what effect variations in their concentration have on milk secretion.

5. In a few very recent experiments attempts have been made to determine the relation between milk secretion and the vitamins of the food.

<sup>1</sup> Published with the permission of the Secretary of Agriculture.

It is of course impossible, in a brief review, to discuss adequately all the experiments that come under the above headings. This article will concern itself chiefly with that part of the field which bears on the problem of the regulation of milk secretion through the diet, taking into consideration particularly the newer work which opens the road toward showing how this regulation is accomplished through changes in the composition of the blood.

I. EXPERIMENTS IN WHICH THE MILK YIELD WAS STUDIED WHILE CHANGES WERE MADE IN THE FOOD. Eckles and Palmer studied the composition and yield of milk in cows which were on generally superabundant rations (24) and on rations generally insufficient to supply their requirements for maintenance and milk yield (25). The results of the experiments with superabundant rations indicated that the milk yield was little or not at all increased by feeding rations more liberal than those required by the accepted standards for feeding dairy cows, and that the composition of the milk was not affected.

The experiments with inadequate rations were carried out partly on cows in the early stage of lactation and partly at a later stage. The cows in the early stage of lactation showed a remarkable power of secreting milk on very inadequate rations. That they used the materials of their own bodies to supply the deficiency was shown by their decreases in body weight. At later stages of lactation the response to inadequate rations consisted more in a reduction of milk yield and less in the mobilization of the body tissues.

The inadequate rations produced no change in the lactose content of the milk.<sup>2</sup> The changes in the concentration of the fat and protein were rather irregular, but the fat content of the milk was increased rather more often than decreased by the inadequate feeding.

Basch reviewed the subject of milk secretion in 1903 (6). He refers to the earlier investigators who studied the effects of changes in particular constituents of the diet on the composition and quantity of the milk yielded, and gives an outline of their results. For citations of work prior to 1903, the reader is referred to Basch's article.

The results of these earlier investigations may be summed up in a few words. There is a general agreement that the quantity of milk yielded is highly dependent on the quantity of protein supplied in the food. It is a very general rule that increased protein in the food results in an increased milk yield, and vice versa. The effects of changes in

<sup>2</sup> Throughout this article the word "content" will be used as above to mean "concentration," not the total amount of the constituent in question secreted.

the quantity of food protein on the composition of the milk are not constant. An increase in the food protein may have no effect on the concentration of milk nitrogen, or may be followed by either an increase or a decrease in this factor. It may similarly be followed by no change or by either of the two possible changes in the concentration of milk fat. But an increase in the quantity of food protein usually results in an increase in the total amount of milk fat secreted.

The results obtained by changing the quantity of fat in the ration are even more variable than those in regard to protein. Some authors find that an increase of fat in the food is followed both by an increased milk yield and by an increased concentration of milk fat; others get neither of these results; still others get one without the other.

Comparatively little work has been done on the effect of changes in the carbohydrate of the food. It has been found, however, that where moderate amounts of carbohydrates are added to or subtracted from the ration, little or no change follows in either the composition or quantity of the milk.

The results which have just been summed up must be considered in relation to the possibility of the conversion of one food constituent into another. Milk protein must, of course, come ultimately from the protein of the food. But many investigators, whose work has been reviewed by Lusk (76, pp. 223-247) have shown that food protein may be converted into either fat or carbohydrate in the animal body; and Jordan and his collaborators (67), (68) have shown specifically that the carbohydrate of the food may be converted into milk fat.

It is easy to see, therefore, why the protein of the food should occupy a dominant position in regulating milk yield. If the food protein is insufficient for the quantity of protein that is being excreted in the milk, the animal must either take protein from her own body or reduce the secretion of milk protein; it is not surprising that she usually does both at the same time. In the cases of fat and carbohydrate, on the other hand, there is a much larger number of possibilities.

It is not surprising, in view of these considerations, that the results of changes in diet on the composition of milk have been rather variable. The effect on milk yield of any change in diet will depend on a great number of accessory experimental aspects—on the amount of the change, on the level at which the change is made, on the quantity of other constituents being fed, on the nutritive state of the animal, and on other factors which the reader can easily imagine. The more modern investigations have kept these factors more or less in view.

Shortly after the publication of Basch's review, Morgen and a number of collaborators attacked the problem of the effect of food on milk yield (89), (90), (91), (92). These authors carried out an extensive set of investigations on the effects of diet on the composition and yield of milk, using sheep and goats as their experimental animals. Their results may be summarized as follows:

When the fat in the ration of a milking animal amounts to less than 0.5 gram daily per kilogram of animal, an addition of fat to the ration, as a substitute for carbohydrate, has a marked effect in increasing the percentage of fat in the milk, as well as the total milk yield. The milk yield and the fat content of the milk continue to increase with the addition of fat to the ration up to about 1 gram per kilogram of animal. The further substitution of fat for carbohydrate has no effect on milk yield, except in unusual cases. When the milk yield is increased by the addition of fat to the ration as described above, the nitrogen content is generally reduced.

Additions of protein to the ration at almost any level tend to increase the milk yield. The nitrogen content of the milk is sometimes increased and sometimes decreased; the fat content is generally lowered, but the total amount of fat secreted is generally increased.

The authors did not try in any of their experiments to use a basal ration in which the carbohydrate was reduced to a minimum. They did, however, try adding moderate quantities of carbohydrate to rations which already contained the usual proportion of that material, and found that it had little or no effect on either the composition or the yield of milk.

The concentration of lactose in the milk was not significantly affected by any of the changes in ration which they carried out.

In recent experiments Cary has studied the effects on milk yield of making large reductions in either the protein or the carbohydrate of the food, starting from an approximately normal ration (15). A large reduction in either of these constituents of the food is followed by a marked falling off in milk yield which begins a few hours after the food is changed. A shortage of protein in the food reduced the nitrogen and fat contents of the milk, the sugar content remaining unchanged. A marked shortage of carbohydrate in the food also reduced the nitrogen content of the milk, but in this case the fat and sugar contents remained unchanged.

Fingerling (33) has carried out experiments on goats in which the quantities of calcium and phosphorus in the rations were varied while

the yield and composition of the milk were studied. Reduction in the calcium and phosphorus of the rations at first produced no effect on the milk yield; after several weeks the yield of milk began to fall off progressively while the concentrations of calcium and phosphorus in the milk rose; these changes were largely reversed by restoring calcium and phosphorus to the rations.

Jordan, Hart and Patten (69) carried out two experiments in which the phosphorus content of the ration was varied while the yield and composition of milk were studied. The phosphorus concentration in the milk was not affected, but the percentage of milk fat was noticeably reduced on the low phosphorus ration, and vice versa. The milk yield was little changed by the change in the food phosphorus; it tended to be a little higher on the low than on the high phosphorus ration.

The experiments of Fingerling and of Jordan, Hart and Patten show that milk yield is not affected by changes in the calcium and phosphorus content of the diet until after a considerable interval. Recent experiments of Meigs and Woodward (84a) indicate that the full effects on milk yield of dietary deficiencies in one or both of these elements may not be exhibited for several years, and that the yield of any given lactation period may depend to a considerable extent on whether or not the lactating animal received sufficient calcium and phosphorus in the rations preceding the beginning of that period.

The results which have just been given may be summarized as follows:

Both the milk yield and the composition of the milk may be considerably influenced by changes in the ration. Of the three main organic constituents of the milk, the carbohydrate is by far the most constant; its concentration is not affected by any of the changes in ration so far studied. Reductions in the protein of the ration result very generally in reductions in the milk yield. The nitrogen content is usually either decreased or unaltered, and the fat content may be altered in either direction or may remain unchanged. Reductions in the fat of the ration have little or no effect on the composition and yield of milk until the amount of fat fed falls below 1 gram per kilogram of animal. When it falls below this level, the milk yield and the fat content of the milk are generally reduced, while the nitrogen content is increased. Small changes in the amount of carbohydrate fed, comparable in absolute magnitude with those which have been studied in the cases of protein and fat have no immediate significant effect on either the yield or the composition of the milk. Large reductions,

amounting to 50 per cent of the total nutrients contained in the ration, reduce the milk yield and the concentration of nitrogen in the milk, while leaving the fat and carbohydrate content practically unchanged. The effects of changes in the amounts of carbohydrate fed when the total quantity of this constituent in the basal ration is reduced to a minimum, have not been studied.

A reduction in the amount of phosphorus in the ration may have the effect of reducing the fat content of the milk, while leaving the phosphorus content and the total milk yield practically unchanged. Simultaneous marked reduction of the calcium and phosphorus in the ration results after an interval in a reduction in the milk yield. The calcium and phosphorus contents of the milk are actually increased, though the total amounts secreted are reduced. The full effects on the milk yield of a shortage of calcium or phosphorus in the ration may not show themselves for a very long time.

The above results are, in many cases, not such as would be expected from a cursory consideration of the subject. In some cases they suggest hypotheses regarding the nature of the relation between food supply and milk secretion; in others they hint at metabolic relations which demand careful consideration.

It may first be pointed out that there is a rather sharp contrast between the effects of changing the organic constituents of the ration and those of changing the inorganic constituents. If either the protein or the total nutrients of the ration be sharply reduced, the milk yield generally begins to fall off within a few hours. In Fingerling's experiments, on the other hand (33), although the relative reduction in the amount of calcium and phosphorus fed was larger than the reductions in the organic constituents studied by the other authors, nevertheless the milk yield was not much affected for several weeks. Fingerling followed the calcium and phosphorus balances in his experiments, and found that his animals began to lose these elements from their bodies, as soon as they were put on the low mineral rations. His results give an interesting picture of the quantitative relations for such phenomena, the rates at which calcium and phosphorus are lost from the bodies of milking animals on deficient rations, the time at which milk yield begins to be affected, the manner in which it is affected, and the extent to which it may be restored by a return to adequate rations.

There are numerous other experiments which show that milking animals may remain for considerable periods in marked negative calcium and phosphorus balance without showing much drop in milk yield.

Among these those of Hart and of Forbes and their collaborators are particularly to be mentioned (52), (42), (43), (44).

The milk yield is much more immediately affected, therefore, by a serious shortage of the organic constituents of the ration than by a shortage of calcium and phosphorus. But the reader must not take away the impression that the milking animal adjusts herself to a shortage of protein, fat or carbohydrate merely by reducing the quantity of the missing material secreted in the milk. The work of Hart and his collaborators (53), (54), (55), (56), (57) shows that cows on an inadequate protein ration go into negative nitrogen balance and fall off in their milk yield at the same time. The work of Jordan and his collaborators, on the other hand (67), (68), shows that cows on an inadequate fat ration may respond by manufacturing fat from carbohydrate as well as by reducing the quantity of milk fat secreted. And there is no doubt that the body stores of fat and carbohydrate are frequently called on when the necessity arises. The situation may be summed up by saying that a serious shortage in any of the food constituents so far studied either throws the milking animal into immediate negative balance as far as that constituent is concerned, or causes her to begin manufacturing the constituent from some other material. In the cases of protein, fat and carbohydrate, however, a serious shortage also has the effect of immediately reducing the amount of milk secreted, whereas a serious shortage of calcium and phosphorus may not affect milk secretion for a considerable time.

The work considered so far indicates that protein plays a predominant part in regulating milk secretion. The authors quoted above frequently make the statement that protein "stimulates milk secretion," and the truth of the statement is borne out by the following aspects of the results. In the first place it is shown by the work of Morgen and his collaborators (90), (91), (92) that changes in the amount of protein fed are likely to affect the milk yield at almost any level of protein feeding, and that the changes in total milk yield produced are large in comparison to those produced by comparable changes in the quantity of either fat or carbohydrate fed. In the second place it comes out in all the pertinent work, but particularly again in that of Morgen and his collaborators, that changes in the quantity of protein fed have a marked effect on the whole milk yield, with subordinate effects on the composition of the milk. Changes in the amount of fat fed, on the other hand, if they have any effect at all, produce comparatively large changes in the fat content of the milk and only moderate changes in the total milk yield.

It is true that large changes in the amount of carbohydrate fed (which mean also large changes in the total energy of the ration) have marked effects on milk yield. But Cary (15) has brought out the suggestive point that a reduction in the carbohydrate of the ration causes a reduction in the nitrogen content of the milk. The significance of this will be much clearer after it has been possible to take up another phase of the subject.

There are other points besides this in the investigations so far considered which call for further research. Why, for instance, should the calcium and phosphorus content of the milk be actually increased when the food content of the same elements is diminished? Why should the amount of phosphorus in the ration have an effect on the secretion of milk fat? Finally, how do changes in the quantity of this or that constituent of the food act on the mammary gland to regulate milk secretion?

The reader will hardly hope for complete and satisfactory answers to these questions. But the most promising method of approach is obviously to go deeper into the chemical changes undergone by the food materials within the body of the milking animal. A number of investigators have thrown light on this field by determining the precursors of the milk constituents in the blood, and the work along this line will next be taken up.

## II. THE PRECURSORS OF THE MILK CONSTITUENTS IN THE BLOOD.

1. *The precursor of lactose.* In 1884 Bert reported experiments (7) which bear on the problem of the precursor of milk sugar. He removed the mammary glands of goats; then had the animals bred and allowed them to give birth to their young. He examined the urine of the mothers just after the young were born and found that it contained sugar while the urine of normal goats did not contain sugar just after the birth of the young. Bert concludes that sugar is thrown into the blood from some source—probably the liver—just after parturition, in order to supply the sugar to be secreted in the milk. When the mammary gland is not present to dispose of the extra sugar, the latter escapes in the urine.

In 1909 Porcher published the results of investigations (96) which confirmed and extended the work of Bert. It was shown that the post-partum glycosuria of females deprived of their mammary glands was accompanied by hyperglycemia; that the sugar in question was glucose and not lactose; and that the post-partum glycosuria persisted for only a short time.

Results rather opposed to those of Bert and Porcher have been reported by Moore and Parker (88), by Marshall and Kirkness (83), and by Foà (34), and have given rise to more or less controversial literature. It seems worth while to give a short discussion of this controversy, as the results of Bert and Porcher lend support to two important propositions in regard to the physiology of milk secretion.

The two propositions are, first, that during lactation dextrose is actively thrown into the blood by some organ, probably the liver; and second, that dextrose is, therefore, probably the precursor of milk sugar. The facts, as observed by Bert and Porcher, are that in goats from which the mammary glands have been removed parturition is followed for a few hours by hyperglycemia and glycosuria. Porcher also found that hyperglycemia and glycosuria followed, for a few hours, the operative removal of the mammary glands from lactating goats. In both kinds of experiments the hyperglycemia and glycosuria remain at their height for less than twenty-four hours and disappear entirely within three or four days. Both Bert and Porcher failed to get these results in guinea pigs, or got them to only a slight extent.

The increased blood sugar and urinary sugar are, therefore, according to the results of these authors themselves, to be regarded as largely in the nature of a significant accident. In goats something happens very soon which either inhibits or disposes of the surplus sugar supply, so that it does not appear in the urine. In guinea pigs, for some reason, the increased blood sugar and urinary sugar do not appear at all.

The results of Marshall and Kirkness, Moore and Parker, and Foà do not really contradict this point of view. Marshall and Kirkness worked only on guinea pigs and obtained the same negative results as Bert and Porcher. Moore and Parker worked on goats, but they are vague about the exact time after parturition at which they examined the urine. In one of the two goats experimented on, the urine did show a small increase in reducing power after parturition. Foà reports only two experiments; in these the mammary glands were removed from lactating goats. The operations each lasted seven hours, and the urine was not examined until three hours after they were completed. As anesthesia and operations are themselves likely to have an effect on blood sugar (100) this form of experiment is less satisfactory than that in which the mammary gland is removed in a preliminary operation and the blood and urine examined after parturition—particularly where the operative procedure takes so long a time as in the experiments just reported.

A more direct method of determining the precursor of lactose in the blood has been devised by Kaufmann and Magne (70). These authors took samples of blood from the jugular vein and from the abdominal subcutaneous vein of cows, approximately simultaneously. The abdominal subcutaneous or mammary vein carries blood coming from the udder (102, pp. 609, 721), whereas blood from the jugular vein may be regarded as equivalent to the blood of the mammary artery, as far as the materials involved in milk secretion are concerned. In milking cows, therefore, it is to be expected that the blood from the mammary vein should contain less of the precursors of the various milk constituents than blood from the jugular vein. Kaufmann and Magne found that in milking cows the mammary blood contained, on the average, about 18 per cent less sugar than the jugular blood: in different experiments the figures varied from 7 to 30 per cent. Kaufmann and Magne controlled these results by repeating the experiment on a dry cow, and found that in this case the samples of blood obtained from the two different sources above mentioned contained equal amounts of sugar. The results of this investigation give strong support to the view that the dextrose of blood is the precursor of milk sugar.

Foà (34), (35) has used still another method to determine the precursors of the various constituents of milk. He removed the mammary glands of sheep, kept them in a vessel full of Ringer's solution at body temperature, and perfused them with Ringer's solution, with various mixtures of blood and Ringer's solution, and with Ringer's solution to which various substances had been added. He determined the quantity of dextrose in the various kinds of perfusion mixtures both before and after they had been caused to circulate through the gland, and determined also the nature of the fluid secreted by the gland when various perfusion mixtures were caused to circulate through it. He found that when a mixture of blood and Ringer's solution was caused to circulate through the gland, milk containing lactose was secreted. At the same time the dextrose content of the perfusion mixture was shown to decrease. The concentration of lactose in the milk secreted could be increased by adding dextrose to the perfusion mixture. When Ringer's solution with dextrose added was used as the perfusion mixture, the gland secreted a watery fluid containing lactose. No lactose was obtained in the fluid secreted by the gland when Ringer's solution alone was used as the perfusion fluid, or with Ringer's solution with galactose added to it. Foà's results taken all together furnish strong evidence for the view that the dextrose of the blood is the precursor of lactose.

My colleague, Mr. C. A. Cary, has thought it worth while to repeat the experiment of Kaufmann and Magne, and has kindly given me permission to report his unpublished results here. Their article on the subject is exceedingly brief, and there is a possibility that if they did not take their samples of mammary and jugular blood strictly simultaneously, their results might have been disturbed by the tendency of the blood sugar to vary as the result of pain, anxiety, etc. (13, pp. 66-80), (100). Mr. Cary has collected samples of jugular and mammary blood from milking cows strictly simultaneously. A cannula is first inserted in the mammary vein and left stoppered until after the jugular cannula has been inserted. Blood is then collected from the two cannulae at the same time. It was found that the mammary blood contained about 24 per cent less sugar than the jugular blood, and the mammary plasma, about 32 per cent less than the jugular plasma.

There is, then, a large mass of evidence showing that lactose is derived from the blood sugar, and there is no contradictory evidence. The controversy between Bert and Porcher, on the one hand, and Moore and Parker, Marshall and Kirkness, and Foà, on the other, may be disregarded as far as this question is concerned. Moore and Parker, Marshall and Kirkness, and Foà claim only that the concentration of blood sugar is not increased after the removal of the mammary gland from milking animals; and there is no necessary connection between this contention and the question whether or not lactose is normally derived from blood sugar.

2. *The precursor of milk fat in the blood.* Foà used his method of experimentation to throw light on the origin of milk fat. He found that when Ringer's solution alone was perfused through the excised mammary gland, a watery solution was excreted which contained no fat (35). But when he perfused Ringer's solution in which olive oil or tri-olein had been emulsified, the gland secreted a watery solution containing fat. The fat secreted was in globules which had the microscopic appearance of globules of milk fat, and it had a lower iodine number than the fat of the perfusion fluid. Foà concludes that milk fat is derived from the triglycerides of the blood.

The author, in conjunction with others, has used the experimental method of Kaufmann and Magne to throw light on the question of the origin of milk fat; but, in order to discuss these experiments, it will be necessary to consider the nature of the phosphorus compounds contained in blood. It is now well established that the great majority of all the phosphorus contained in normal blood plasma is divided

between two classes of compounds—the phosphatids and the inorganic phosphates (1), (2), (46), (47), (48), (99). Our own results indicate that there is no phosphorus of any kind in plasma except these two (84). Bloor (11) finds that a third kind of phosphorus makes up about 4 per cent of the total; and Feigl (27), (28), (29), (30), (31) finds about 6 per cent.

Our experiments above referred to (84) were primarily designed to determine whether the phosphorus of milk was derived from the phosphatid or from the inorganic phosphate of the blood plasma. Samples of blood were collected approximately simultaneously from the jugular and mammary veins of milking cows; the plasma was separated from the corpuscles and analyzed for phosphatid and for inorganic phosphate. When the experiments were carried out without too much disturbance to the animals used as subjects, it was found that the mammary plasma contained less phosphatid than the jugular and more inorganic phosphate. The changes in the two kinds of phosphorus nearly offset each other so that the jugular and mammary plasma contained nearly equal total quantities of phosphorus. When the animals were much disturbed by the experimental procedure, the jugular and mammary plasma contained equal quantities of phosphatid, but the mammary plasma contained more inorganic phosphate, and, therefore, more total phosphorus than the jugular. These results have been interpreted to mean that milk phosphorus and milk fat are derived from the phosphatid of the blood plasma. The phosphatid of the plasma probably contains about one part by weight of phosphorus to 20 parts by weight of fatty acids, while milk contains about one part by weight of phosphorus to 50 parts of fat. If, therefore, the mammary gland takes from the plasma enough phosphatid to supply a given quantity of milk with fat, it gets with it more than twice as much phosphorus as is required for the same quantity of milk. The surplus phosphorus must be returned to the blood, and this is taken to be the explanation of the fact that the mammary plasma regularly contains more inorganic phosphate than the jugular. Disturbance of the cow tends to stop the taking up of phosphatid by the mammary gland, but not the backflow of inorganic phosphate from the gland to the blood. For this reason, the cows which have been much disturbed before and during the collection of the blood samples show an increased quantity of inorganic phosphate in the mammary plasma and no change in the phosphatid content. The experimental results described above will be further considered later, and their relation to the results of Foà on fat secretion and to certain results of Bloor will be discussed.

3. *The precursor of milk protein in the blood.* Foà (35) tried some experiments on the excised mammary gland of the sheep to throw light on the derivation of milk protein, but his results were negative. He perfused the gland with Ringer's solution to which various forms of protein had been added, but never found any protein in the fluid secreted under such circumstances. He concludes that milk protein is derived from some still unknown material in the blood.

The recent epoch-making work on protein metabolism of Delaunay (20), (21), (22), Folin (36), (37), (38), (39), (40), (41), Van Slyke (103), (104) and their followers suggests that milk protein may be derived from the free amino-acids of the blood. Cary has recently employed the experimental method of Kaufmann and Magne to determine this point (14). He collected samples of jugular and mammary blood approximately simultaneously from both milking and dry cows. The amino-acid nitrogen of the mammary blood was markedly lower than that of the jugular blood in the milking cows, whereas the two kinds of blood contained the same concentration of amino-acid nitrogen in the dry cows. The results form very complete and satisfactory evidence for the view that the milk proteins are derived from the free amino-acids of the blood.

4. *General discussion of the precursors in the blood of the various constituents of milk.* Evidence has been adduced to show that lactose is derived from the dextrose of the blood, that milk fat is derived from phosphatid, and that milk protein is derived from free amino-acids. It seems worth while to point out, in the first place, that each of the three propositions stated above gains strength from the other two, when the experimental results on which all three are based are compared quantitatively and in detail; and, in the second place, that the three propositions may be taken together as indicating a sort of hypothesis of secretion which is *a priori* probable, and which suggests an explanation of certain aspects of the subject sometimes regarded in the past as rather mysterious.

Cow's milk is a fluid with a fairly constant composition. Typical milk may be considered to contain 3 per cent protein, 4 per cent fat and 5 per cent lactose. If it may be assumed that in cows giving the same quantities of milk the rate of blood flow through the mammary gland is approximately the same, then the amounts by which the precursors of the three above-named constituents of milk are reduced in the mammary blood ought to bear a more or less fixed relation to each other in the case of cows giving approximately the same quantities of milk.

In the experiments of Kaufmann and Magne, in those of Meigs, Blatherwick and Cary, and in those of Cary, the cows were usually giving about 10 liters of milk daily. Kaufmann and Magne found the sugar in the mammary blood of their milking cows about 15 mgm. per 100 cc. lower than that in the jugular blood. In the unpublished experiments of Cary above referred to, the sugar was determined in mammary and jugular plasma as well as in mammary and jugular blood; and it was found that the sugar taken from the blood by the mammary gland came entirely from the plasma, the sugar content of the corpuscles remaining unchanged during their passage through the mammary gland. The reduction of 15 mgm. per 100 cc. blood found by Kaufmann and Magne would be equivalent, therefore, to a reduction of 22 mgm. per 100 cc. plasma. Cary found a reduction of 20 mgm. per 100 cc. in the mammary plasma.

In the two successful experiments on the phosphatid content of the jugular and mammary plasma by Meigs, Blatherwick and Cary, the phosphatid phosphorus was found to be lower by 0.74 and 0.60 mgm. per 100 cc. respectively in the mammary plasma. This would represent reductions amounting to 14.8 and 12 mgm. of phosphatid in the mammary plasma.

In Cary's experiments on the amino-acid nitrogen of jugular and mammary plasma in milking cows, the amino-nitrogen was found reduced by from 0.39 to 0.97 mgm. per 100 cc. in the mammary plasma, the average reduction being 0.69 mgm. As the alpha amino-nitrogen of the milk proteins constitutes about 70 per cent of their total nitrogen, this would represent a reduction of about 1 mgm. total milk-protein nitrogen per 100 cc. plasma, which would be equivalent to about 6 mgm. of protein.

On the supposition that the milk of the cows used in these experiments contained 5 per cent lactose, 4 per cent fat and 3 per cent protein, the precursors of these three constituents should have been taken out of the plasma passing through the mammary gland in the following proportions. For every 20 mgm. of sugar taken from the plasma there should have been taken phosphorus equivalent to 16 mgm. of fat and amino-nitrogen equivalent to 12 mgm. protein. The figures actually found and given above are 15 to 20 mgm. sugar, phosphorus equivalent to from 12 to 15 mgm. fat, and nitrogen equivalent to from 3 to 9 mgm. protein. The figures for sugar and fat secretion bear very nearly the expected relation to one another, while the figures representing the secretion of milk protein are somewhat lower than would be expected.

But when it is considered that the concentrations of sugar, phosphatid and free amino-acids in blood plasma are all quite low, and that small errors in estimating the actual amounts of these materials present in a given sample would be represented as much larger errors in comparing the differences between jugular and mammary plasma, it seems remarkable that the results actually found should be as close as they are to those that would be expected. Taken together, they furnish strong evidence for the view that the lactose, fat and protein of the milk are respectively derived entirely from the dextrose, phosphatid and free amino-acids of the blood.

The three sets of observations above discussed suggest a working hypothesis of secretion which is *a priori* probable. The mammary gland is called upon to secrete large quantities of protein, fat and carbohydrate at a rapid rate. It is hardly probable, therefore, that it manufactures its products from widely different chemical compounds brought to it by the blood. But it has been clear for some time that casein and lactose, and probably milk fat also, are specific products of the mammary gland. The two former are certainly not present in the blood under ordinary circumstances. Even if the constituents of milk could be demonstrated in the blood it would be difficult to see how the mammary gland could abstract them for the purpose of making milk. Casein and fat are highly indiffusible substances, and milk contains sugar and fat in much higher concentration than does blood. It is very difficult to conceive a mechanism by which such materials could be made to diffuse rapidly from a region of lower concentration to one of higher concentration. But the observations given offer some escape from all these difficulties. To suppose that the mammary gland converts dextrose into lactose, phosphatid into fat, and free amino-acids into casein, leaves undoubtedly many chemical problems still to be solved. But it is conceivable that such changes could take place at a rapid rate and without any great expenditure of energy. Further, dextrose, phosphatid and free amino-acids are more diffusible substances than lactose, triglycerides and proteins; to suppose that the constituents of milk pass the borders of the mammary cells in the form of the three former substances, and are converted, immediately on entering the cells, into the less diffusible latter three compounds, furnishes a rough explanation of the mechanism of secretion, which does not contradict any of the laws of energetics.

The dextrose, the phosphatid and the free amino-acids of the blood plasma are spoken of in this article as the "precursors of the milk con-

stituents." But this is really a one-sided view of them, with no further basis than that milk secretion is the subject of the article. There is little doubt that the three materials above-mentioned are really the general currency of metabolism—the chemical forms in which proteins, fat, and carbohydrate are distributed by the blood to all the organs and tissues of the body.

5. *The relation between blood flow through the mammary gland and milk secretion.* Cary (14) found no relation between the rapidity of milk secretion and the degree of difference in amino-acid nitrogen concentration as between the jugular and mammary blood plasma. Most of his experiments were carried out on cows giving about 10 liters of milk daily; and while there was considerable variation in the degree of difference between jugular and mammary plasma in these experiments, there was no tendency for the differences to be greatest in the cows which were giving the most milk. One experiment was carried out on a cow which was giving more than twice as much milk as any of the others, and in this case the difference in the amino-nitrogen content of the jugular and mammary plasma was practically the same as the average for the other experiments.

This aspect of his results suggests that the milk yield is in general nearly proportional to the rate of blood flow through the udder. The proposition is probable from general considerations and is supported by a good deal of independent evidence. That given by Roehrig (98) is impressive; and it is well known to dairymen and now statistically demonstrated that there is a fairly close correlation between the size of the milk veins and productiveness in cattle (45).

6. *The effects of pain, anxiety, etc., on milk yield.* The experiments of Meigs, Blatherwick and Cary on fat secretion indicate that this process is stopped by an even slight disturbance of the cow's comfort. Cary's experiments on protein secretion, on the other hand, do not show any such relation with regard to this latter process. With regard to sugar secretion, evidence on the point at issue is difficult to obtain, because pain and anxiety have a tendency to change the sugar concentration in the whole body of blood (13, pp. 66–80) (100). But, as far as they go, the experiments on record point to the view that the secretion of milk sugar is not noticeably interfered with by pain and anxiety.

There is some independent evidence indicating that fat secretion is more interfered with by pain and anxiety than the secretion of the other constituents of milk. It is commonly thought by dairymen that the percentage of fat in milk is likely to be reduced when cows are chased

about by dogs or otherwise disturbed, and when they are in heat. I have observed this tendency occasionally, though I have not carried out any special research on the subject.

7. *The opposition between Foà's conclusions and those of Meigs, Blatherwick and Cary.* The results of Meigs, Blatherwick and Cary indicate that milk fat is derived entirely from the phosphatid of the blood plasma. The disappearance of phosphatid from the plasma which occurs during its passage through the mammary gland is sufficient to account for all the milk fat secreted. Further, the very considerable backflow of inorganic phosphate from the gland to the blood would be difficult to account for if it were supposed that any considerable part of the milk fat were derived from non-phosphorized fat in the blood.

The work of Foà, on the other hand, indicates that milk fat may be derived from triglycerides in the blood. Foà found that a fluid containing fat was secreted by the excised mammary gland when it was perfused with Ringer's solution to which olive oil or triolein had been added. That the olive oil and triolein had not simply filtered through the gland cells into the ducts is indicated by the facts that the fat in the fluid obtained from the duct was in the form of globules with the microscopic appearance of those normally seen in milk, and that it had a lower iodine number than the fats mixed with the perfusion fluid. That the fat in the fluid issuing from the duct was not simply fat which had been present in the gland cells before the beginning of the experiment and had been washed out into the ducts by the perfusion fluid is shown by the fact that when Ringer's solution without fat was perfused through the vessels, there issued from the duct a fluid which contained no fat.

The chief objection to Foà's experiments is that, as he himself admits, the gland became edematous quite early in the procedure, and that the collection of the fluid issuing from the duct, which was afterwards analyzed, was continued after the edema had been noted. It seems quite likely that the edematous gland cells would give passage to substances which would not pass through the normal mammary cells. It is true that the fat in the fluid issuing from the duct in Foà's experiments had a different iodine number from that in the perfusion fluid; but it is not unlikely that the bodies of the mammary cells might retain some portion of their capacity to alter the character of fats, even after the cell surfaces had become permeable to materials which would not ordinarily go through them.

III. THE CONTROL OF MILK SECRETION THROUGH THE CONCENTRATIONS OF AMINO ACIDS, DEXTROSE AND PHOSPHATID IN THE BLOOD. The investigations reviewed in section I show that changes in diet often produce marked changes in milk secretion, and they bring up the question how milk secretion is controlled through the diet. There can be little doubt that diet exercised its influence by producing changes in the composition of the blood. What is the nature of these changes?

The hypothesis that occurs first to most minds is that a shortage of any particular dietary constituent will bring about a reduction in the concentration of the corresponding milk precursor in the blood. It is natural to suppose that the result of this change will be a reduction in the concentration of the corresponding constituent of the milk, and perhaps also a reduction in the total amount of milk secreted.

The experiments in which the composition and yield of milk have been followed while changes were made in the diet furnish evidence for thinking that the above outlined hypothesis is correct in the case of fat. Reduction in the dietary fat below a certain level produces a marked decrease in the concentration of milk fat and, at the same time, a considerable reduction in the total quantity of milk secreted (89), (90), (91), (92). But in no other case is the evidence so clear. Changes in the quantity of dietary protein produce changes in the quantity of milk secreted through a wide range in the level of protein feeding (90), (91), (92), but the accompanying changes in the concentration of milk protein are irregular. The lactose content of milk is not noticeably influenced by any of the dietary changes so far studied. And the concentrations of calcium and phosphorus in milk may be actually increased by feeding a ration short in these elements (33).

Further light on the question of the control of milk secretion through the diet must be sought in a study of the manner in which the concentrations of the several milk precursors in the blood are affected by various circumstances. The most satisfactory way of studying the question is to follow simultaneously the concentrations of the milk precursors in the blood and the composition and yield of milk while appropriate changes are made in the diet. But few such experiments have been carried out up to the present time; and, while waiting for their number to be increased, it is pertinent to consider the considerable quantity of work already at hand which bears on the general question of the factors which influence the concentration of amino-nitrogen, glucose, phosphatid and calcium in the blood.

Blood is a complicated mixture containing many unstable chemical compounds. It is not surprising, therefore, that the literature contains contradictory figures for the concentration of practically every blood constituent which has been studied. In estimating the value of these results, the following considerations must be kept in mind.

If the concentration of a given constituent of the blood is really constant, it is easy to see how inadequate chemical methods for its determination might make it appear variable. But if the concentration of a given constituent is really variable, it is extremely difficult to see how inadequate chemical methods could make it appear constant. Other things being equal, therefore, the work of those investigators who obtain constant series of results is to be accepted in preference to that of those who obtain more variable series.

In cases where several independent investigators have obtained constant series of results all agreeing closely with one another, the case becomes stronger; and it is justifiable to summarily dismiss non-concordant results obtained by other investigators, unless these latter can show why their methods should be preferred to those of their opponents.

This course will be adopted in the following discussion. In such a review as this it is impossible to give either a critical discussion of the very complex methods of blood analysis or a detailed account of the great mass of wild results which have been obtained in the field. Only those figures will be considered, therefore, which, in the light of our present knowledge, appear to be fairly reasonable.

1. *Relation between milk secretion and the free amino-acids of the blood.* An admirable review of the modern work on protein metabolism has recently been published by Van Slyke (105). The experimental results show that the food protein is converted in the intestinal tract to free amino-acids, in which form it enters the blood. The blood from the intestinal tract passes first to the liver, and in that organ a considerable proportion of the amino-acids resulting from protein digestion is deaminized. The remainder goes on into the general circulation, serving, for some time, to increase the concentration of amino-nitrogen in the systemic blood, but being gradually absorbed by the tissues and either built into new protein or deaminized and oxidized.

Still more recently Cathcart has published a new edition of his monograph on the physiology of protein metabolism (16), which contains an extensive bibliography.

In section I work was reviewed which indicates that milk yield is highly dependent on the protein supplied in the food; section II contains

work which shows that milk protein is derived from the free amino-acids of the blood. It seems natural, in view of these facts, to suppose that milk yield is largely regulated by changes in the amino-acid mixture circulating in the blood.

An obvious method of investigating this problem is to follow the milk yield and the amino-nitrogen content of the blood, while changes are made in the diet. But such experiments cannot be intelligently planned without taking into consideration the various aspects of metabolism which are likely to influence the results.

The blood carrying the products of digestion from the intestinal tract passes first to the liver, and the results of Van Slyke (105) indicate that that organ immediately begins to deaminize some of the amino-acids which it receives. But we know very little in regard to the details of this hepatic activity. As the amino-nitrogen content of the blood is so small, and as so large a proportion of it is removed by the mammary gland in milking animals (14), it is natural to suppose that the liver's deaminizing activity is more or less selective—that it improves the quality of the amino-acid mixture in the general circulation by deaminizing chiefly those compounds which are present in the food in larger proportion than is required for milk secretion, tissue growth or other metabolic activities. But this point of view is by no means strictly proven; and, even if it were, it would still be possible that a marked change in the quality of the protein supplied in the food might produce a less marked but still important change in the quality of the amino-acid mixture circulating in the blood.

The quality of the amino-acid mixture in the blood might be influenced in another way, which is particularly likely to occur in milking animals; namely, by a change from a positive to a negative nitrogen balance. In the case of an animal in positive nitrogen balance, the amino-acids which enter the general circulation pass first through the liver. But if the nitrogen balance becomes negative, amino-acids are thrown directly into the general circulation by the tissues, and may not reach the liver until after the particular portion of blood in which they are contained has circulated a number of times. It is quite possible that such circumstances might produce considerable changes in the quality of the amino-acid mixture circulating in the blood.

Finally, the deaminizing activity of the liver is probably influenced by the supply and demand of nutritive materials other than protein. In the case of a milking animal there is a large and steady demand for carbohydrate to be excreted with the milk. The supply of carbohydrate

stored by mammals is normally rather small, and a shortage of this material in the food would, therefore, soon require the conversion of other compounds into carbohydrate to meet the demands of milk secretion. It has been repeatedly shown that carbohydrate is readily derived from protein in the animal economy (76, pp. 223-247), and it is very likely, therefore, that a shortage of carbohydrate in the food of a milking animal would soon stimulate the liver to increase its normal rate of deaminizing the products of protein digestion.

The three preceding paragraphs may be summed up by saying that general considerations make it likely that the quality as well as the quantity of the amino-acid mixture circulating in the blood is subject to change, and that both quality and quantity of the circulating amino-acid mixture are likely to be influenced by the supply of non-protein dietary constituents. There is already at hand a certain amount of experimental evidence which bears on these questions.

Delaunay (22), Van Slyke and Meyer (103), (104), Costantino (18), and György and Zunz (49) have shown that, during the digestion of a protein meal, the concentration of the amino-acid nitrogen in the blood is usually decidedly higher than when the intestinal tract is empty after 1 or 2 days' starvation. The earlier work of Folin and Denis (36), (37), (38), (39), (40), (41) shows the same thing, though these authors did not make any direct determinations of amino nitrogen.

Bang (4) has published results which lead him to conclude that the amino nitrogen of the blood is not, as a rule, increased by feeding protein. But the figures which he gives as representing amino nitrogen are really obtained by subtracting the urea nitrogen from the total non-protein nitrogen of the blood (4, p. 105), and, on account of this and of their general irregularity, they should hardly be taken as contradicting the mass of opposing evidence based on more direct methods.

Van Slyke and Meyer (104) have published figures for the amino nitrogen of the blood during several days' starvation. The results are rather irregular, but indicate, on the whole, that there is no marked decrease in the amino nitrogen of the blood during starvation continued for more than one or two days.

The work of Folin and Denis (36), (39) gives strong reason for believing that the quality of the amino-acid mixture contained in the blood of the general circulation is subject to alteration. These authors introduced various single amino-acids into the intestinal tract, and found that there was soon afterward a marked rise in the nitrogen fraction obtained by subtracting the urea nitrogen from the total

non-protein nitrogen of the blood. It would be difficult to explain this result without supposing that the blood obtained during the absorption of the single amino acid contained much more of this latter body than was normal.

Three rather elaborate experiments have been carried out by Cary to throw light on the changes produced in the concentration and quality of the amino acid mixture of the blood by various kinds of changes in the diet of milking cows, and also on the manner in which milk yield is affected by changes in the amino acids of the blood. Abstracts of this work have already been published (15), and Mr. Cary has kindly given me permission to discuss the work further in this article.

All three experiments were begun with cows which were giving liberal quantities of milk, and which were on rations sufficient to provide for their maintenance and milk yield according to the figures given in the standard textbooks. Under these conditions, the composition and quantity of the milk and the concentration of amino-acid nitrogen in the blood and plasma were determined. The animals were then changed to rations which were inadequate in various respects, kept on these for varying periods, and finally changed back to the original adequate rations. Through all these changes, the composition and quantity of the milk and the concentration of amino-acid nitrogen in the blood and plasma were determined.

In the first experiment, the inadequate ration used contained only about half the required quantities of protein and total nutrients. In the second experiment, it contained half the required quantity of protein, but carbohydrate was substituted for the protein removed, so that it still contained the full requirement in total nutrients. In the third experiment, the inadequate ration contained the full requirement in protein, but only about half the original quantity of total nutrients. The change was accomplished chiefly by removing carbohydrate from the ration.

It will be possible, here, to give the results only in a very general way; those of the third experiment will be considered first. In this case, the reduction in the carbohydrate of the ration caused a reduction in milk yield, reductions in the amino-nitrogen content of the blood and plasma, and a reduction in the nitrogen content of the milk. The fat and sugar content of the milk remained practically unchanged. The results indicate that a shortage of carbohydrate in the food affects the milk yield through changes brought about in the amino-acid mixture of the blood.

It is not really surprising that this should be the case. A milking cow must have a large supply of carbohydrate daily to put into her milk. This material must come either from the carbohydrate of the food, from carbohydrate stored in the body, or from the conversion of some other material into carbohydrate. The available carbohydrate store is always small, probably not enough to supply a moderate milk yield for more than a few hours. When, therefore, the carbohydrate in the food of a milking cow is severely cut, the animal must soon begin to manufacture the missing material from something else. It seems quite reasonable to suppose that under such circumstances the liver would be stimulated to deaminize more rapidly the amino acids carried to it in the portal blood, and thereby to supply the missing carbohydrate and to reduce the concentration of amino nitrogen in the general circulation.

In view of the results of the experiment just described, it is not surprising to find that in the first experiment where the protein and total nutrients of the ration were reduced together, there was a very marked reduction in the amino-acid nitrogen of the blood, in the milk yield, and in the concentration of milk nitrogen. The milk sugar content was not determined in this experiment; the concentration of milk fat showed a general tendency to rise a little.

In the second experiment the quantity of protein in the ration was reduced, while the quantity of total nutrients remained unchanged. But, in this case, 50 per cent of the protein in the original adequate ration consisted of casein and lactalbumin, and 50 per cent consisted of vegetable protein. The reduction was made by leaving out all the casein and lactalbumin; and, as a consequence of this, the protein in the inadequate ration was not only reduced in quantity but also decidedly inferior in quality, as far as its relation to milk secretion was concerned. The result of the change in diet was that the quantity and nitrogen content of the milk were reduced, while the concentration of amino-acid nitrogen in the blood remained unchanged. In this experiment again, the sugar content of the milk remained unchanged on the inadequate diet. The fat content was decidedly decreased.

It is probable that the changes in quality of the food protein made by the change in diet brought about a corresponding change in the quality of the amino-acid mixture of the blood, and that this change produced the lowered milk yield and the lowered concentration of milk nitrogen, in spite of the fact that the concentration of total amino-acid nitrogen in the blood remained unchanged.

In a more recent experiment, the protein in the diet was reduced without changing its quality. In this case the same effects were obtained on milk yield as in the preceding experiment, and the concentration of amino-acid nitrogen in the blood was decidedly reduced.

When the animals in the experiments described above were changed back to the adequate rations, the changes in the milk yield and in the concentration of amino-acid nitrogen in the blood were not the exact reciprocals of those which were obtained by the change from the adequate to the inadequate rations. The reason for this is probably that the inadequate rations induced negative nitrogen balances, and that the animals were, therefore, in quite different nutritive condition at the beginning and end of each period on inadequate rations. It is unfortunately impossible to give any discussion of this aspect of the experiments here.

The most important general conclusions to be drawn from the experiments which have just been described are that both the quantity and the quality of the amino-acid mixture circulating in the blood are changed by changes in diet, that these changes have a marked influence on milk secretion, and that protein metabolism is intimately related to the metabolism of both carbohydrate and fat. A change in the carbohydrate of the diet may affect the amino-acids of the blood, and thereby the secretion of milk protein; and a change in the protein of the diet may affect the secretion of milk fat—probably again through a change in the amino-acids of the blood.

2. *Relation between milk secretion and the carbohydrate, phosphatid and calcium of the blood.* No experiments have been carried out in which the milk yield and either the dextrose, phosphatid or calcium of the blood have been studied simultaneously. But there is a good deal of evidence in regard to how the concentration of these three constituents of blood varies under various physiological conditions; and, also, as to how variations in the quantity of carbohydrate, fat, phosphorus and calcium in the food affect the milk yield. Bringing these two sets of investigation into relation with each other throws a good deal of light on the physiology of nutrition and of milk secretion.

The concentrations of both dextrose and phosphatid in the blood are subject to considerable variation. The blood sugar content may vary with the quantity of carbohydrate supplied in the food (77, p. 223), and is also altered by such influences as anxiety, pain and anesthesia (13, pp. 66-80) (100). The concentration of milk sugar, on the other hand, is surprisingly constant (89), (90), (91), (92), (25), (15). None

of the investigations so far carried out throws any light on the question whether milk secretion is affected by changes in the concentration of blood sugar. In Cary's investigation (15) the milk yield was found to decrease when the carbohydrate in the ration was greatly decreased. But Cary's work shows that a reduction in the carbohydrate of the food causes a reduction in the concentration of amino nitrogen in the blood and also a reduction in the concentration of the milk nitrogen. It is quite probable that changes in the amount of carbohydrate supplied in the food, and changes in carbohydrate metabolism generally, affect the milk yield through changes which they induce in protein metabolism rather than through any change brought about in the concentration of carbohydrate in the blood. The same comment would apply to a number of researches in which the effects of phlorhidzin administration on milk yield and on the concentration of milk sugar were studied (17), (19), (93), (94), (95).

The concentration of phosphatid in the blood has been shown to be increased by feeding fat (9), (10). It is also increased during milk secretion independently of the food supply (84). The work of Morgen and his collaborators (89), (90), (91), (92) shows that the concentration of milk fat may be influenced by the fat of the food; that of Jordan, Hart and Patten (69), that it may be influenced by the phosphorus contained in the food. It seems likely that changes in the quantity of fat or phosphorus contained in the food influence the secretion of milk fat by bringing about changes in the concentration of phosphatid circulating in the blood; but as yet no direct proof of this proposition has been adduced.

The concentration of calcium in blood plasma is more constant than that of any other constituent which has been hitherto studied. In adult human beings and cattle the normal limits are from 9 to 11 mgm. per 100 cc. plasma (51), (64), (66), (72), (73), (74), (82), (84), (87). The changes in concentration which can be brought about by changing the quantity of calcium in the food of these animals are barely outside the limits of error for the determinations (8), (50), (84). It is not surprising, therefore, to find that reductions in the amount of calcium in the food, even when sufficient to bring about a marked negative calcium balance, have no immediate effect on milk secretion (33), (42), (43), (44), (52).

It will be seen from the foregoing paragraphs that our knowledge of the manner in which diet affects milk secretion through changes brought about in the concentrations of the various milk precursors in the blood is still in a very fragmentary state. The evidence now on hand seems to justify the following views.

Milk secretion is preëminently affected through changes in the quality and quantity of the amino-acid mixture circulating in the blood. Such changes are brought about by changes in the quantity or quality of the protein fed, and also by marked changes in the quantity of the non-protein portion of the ration. They tend to affect the whole amount of milk yielded rather than the concentration of protein in the milk, though the latter kind of change can easily be detected when the experimental conditions are appropriate. The amino-acid mixture circulating in the plasma seems, under certain circumstances, to have an effect on the secretion of milk fat.

In the experimental procedures hitherto used the effects on milk secretion of changes in carbohydrate metabolism are confused with the effects of changes induced in protein metabolism. It is improbable that milk secretion is affected by any changes in the concentration of blood sugar which can be brought about under ordinary circumstances.

Milk fat is derived from the phosphatid of the blood plasma. Its secretion is probably, to some extent, dependent on the concentration of phosphatid in the blood, and therefore on both the fat and phosphorus supplied in the food. That milk fat is usually derived from food fat is shown not only by the work of Morgen and his collaborators frequently referred to above, but also by numerous investigations in which it has been shown that the quality of the milk fat is influenced by the quality of the food fat and that fatty acids not normally contained in butter can be made to appear in it if they are supplied in the food. As examples of the evidence for these two propositions, the work of Eckles and Palmer (26) and of Bowes (12) may be referred to. These authors give references to previous work on the subject. The connection between the secretion of milk fat and the phosphorus of the food is shown by the work of Jordan, Hart and Patten (69).

The work of these authors shows that the concentration of milk phosphorus is not affected by the phosphorus supplied in the food, though the concentration of milk fat is affected; and the reason for this rather peculiar situation is given in the work of Meigs, Blatherwick and Cary (84). The phosphatid of the blood contains more phosphorus in proportion to fat than does the milk. In taking sufficient phosphatid from the blood to provide for the milk fat, therefore, the mammary gland always gets more phosphorus than is required to go into the milk, and it returns the surplus to the blood as inorganic phosphate. It is quite conceivable that the return of this extra phosphorus to the blood might be independent of the amount of phosphorus contained in the

food, and that the concentration of phosphorus in the milk might, therefore, be independent of the food phosphorus.

The calcium contained in milk must be derived from the calcium of the blood plasma or from some part of it. The plasma calcium may, therefore, be regarded as the precursor of the milk calcium. The concentration of plasma calcium is extremely constant and largely independent of the food supply. It is not surprising, therefore, to find that changes in the quantity of calcium in the food have no immediate effect on milk secretion. A long-continued deficiency of calcium and phosphorus in the food does finally bring about a reduction in milk secretion, as has been shown by Fingerling (33). But the same author shows that, as the total yield of milk falls off, the concentration of calcium and phosphorus therein rises; and it seems quite possible that a deficiency of calcium in the diet may affect milk yield in some indirect way, and not through a change in the calcium content of the blood plasma.

IV. QUANTITATIVE ESTIMATIONS OF THE AMOUNTS OF PROTEIN AND ENERGY REQUIRED FOR MILK SECRETION. An account of the relation between milk secretion and diet would be incomplete without a brief review of the attempts which have been made to determine the quantities of protein and total nutrients or energy required to support a given amount of milk secretion in cows.

Accounts of the work along these lines and references to the literature are given in the standard textbooks on cattle feeding (3), (60), (71), (75), and tables are published in which it is stated that a cow of a given weight requires a certain quantity of protein and a certain quantity of total nutrients for her maintenance, and, in addition, so much protein and so much nutrients for each pound of milk with a given fat content.

It is clear from a study of the original work on the subject that the aim has been to determine the quantities of protein and nutritive energy necessary to keep a cow in nutritive equilibrium when she is giving a certain quantity of milk. The difficulties in the way of making such determinations are very considerable. It requires, in the first place, an elaborate and expensive experiment to determine whether an animal is in nitrogen equilibrium or in energy equilibrium: many of the investigators of the subject have not attempted to do this accurately, but have merely used the body weight as a rough indication as to whether their animals were in nutritive equilibrium or not. Further difficulties are introduced by the natural variation in the qualities of foods, and in the different proportions of the same digested by different

animals or by the same animal under different conditions. In the case of protein, finally, there is, strictly speaking, no such thing as a quantitative requirement for milk secretion at all. The milk proteins contain certain amino-acids, such as tryptophane and lysine, which cannot be manufactured from other materials by the animal body and which are contained in varying amounts in certain vegetable proteins and are absent altogether from others (105, pp. 70 et seq.). The quantity of protein required to support a given milk yield will vary, therefore, with the quality of the protein supplied; and it is obvious that the figures given as the protein requirement for milk secretion cannot be taken as generally accurate for all kinds of protein. They represent, at best, the requirement for milk secretion of the particular kind of protein used in the experiment in which they were obtained.

In spite of all these difficulties, the figures obtained by different investigators do not vary so widely as might be supposed. As was to have been expected, the figures given for protein requirement are the most variable. It is difficult to make a thorough-going comparison of all the figures, because the different investigators give their results in different terms which are not strictly comparable. In a general way, however, it may be said that the difference between the high and low figures given for protein requirement amounts to about 33 per cent of the latter; while the same difference in the case of total nutrients or energy amounts to about 13 per cent (3, p. 714; 60, pp. 133 and 667). If the figures for the requirements are compared with the products obtained in the milk, it will be found that the production of a gram of protein or of total nutrients in the milk requires about twice that weight of protein or of total nutrients in the food, in addition to the maintenance requirement (60, p. 133).

The optimum yield of a milking animal is often considerably above that which she will give when kept in nutritive equilibrium. A clear discussion of this phase of the subject is given by Armsby (3, pp. 513 et seq.). If fed more than is required to preserve nutritive equilibrium, the lactating animal will put part of the surplus into an increased energy output, part into body growth, and part into increased milk yield. Just what proportion of the increased food intake will take each of these three outlets under different circumstances is a question of very great practical importance, but there are considerable difficulties in the way of its investigation, and it has so far hardly been touched.

V. VITAMINES AND MILK SECRETION. The question of the relation of vitamins in the diet to milk secretion has, as yet, been little studied.

The whole subject is too new to make possible any very detailed discussion or definite conclusions, but there are not wanting results which point to its importance, and these will be briefly referred to. An extensive review of the general subject of vitamins in nutrition is given by Sherman (100a).

It is probably a mistake to suppose that the three little known materials which at present occupy the center of the stage under the name of vitamins are the only unknown chemical compounds which are required for the nutrition of all animals under all physiological conditions. We already have hints of the existence of one or two rivals, and it is altogether probable that a good many more will make their appearance as soon as investigators begin to search for them. For some time past evidence has been in existence which indicates that there are compounds which have an influence on milk secretion in addition to those generally recognized.

The evidence in question comes from the work of Morgen and his collaborators (32), (91). These investigators found that when milking animals were fed on a basal ration of factory products, their milk yield was noticeably increased by the addition of small quantities of what they call "Reizstoffe"—certain aromatic seeds and the water extract of meadow hay. The basal rations in these experiments were probably low in all three vitamins, and the beneficial effects on milk yield of adding the "Reizstoffe" may have been due to the addition of either the water-soluble or the fat-soluble vitamin, or of both. It is possible, on the other hand, that they were due to some material not identical with any of the three vitamins which are receiving so much consideration at present.

According to McCollum (79), the fat-soluble vitamin is generally represented fairly well in the leaves of plants, while the water-soluble is everywhere plentifully present in seeds. Most cattle rations composed of grain and hay ought, therefore, to contain these two vitamins.

It seems likely, however, that milking animals kept on the usual winter rations would get very little of the antiscorbutic vitamin in their food. Barnes and Hume (5) have reported that the antiscorbutic property of cow's milk varies with the season, and Hart, Steenbock and Ellis (58) have published experiments showing that the milk from cows on pasture contains more of the antiscorbutic vitamin than that from cows fed exclusively on winter rations. These results have been confirmed by Hess (62) and by Dutcher and others (23). They indicate that the antiscorbutic vitamin is not synthesized by milking animals,

and that its absence from the food reduces its concentration in the milk rather than the total milk yield.

McCollum, Simmonds and Pitz (78), (80) have described experiments which indicate that the growth of young nursing rats is retarded when the fat-soluble and water-soluble vitamins are absent from the diet of their mothers. The authors believe that this is due to a reduced concentration of the vitamins in the milk rather than to a reduction of the total milk yield, but they adduce no experimental evidence in support of their belief. Dutcher (23), however, and Hughes (65) have announced that in experiments to be published later they have shown that it is possible to reduce the concentration of either the fat-soluble or the water-soluble vitamin in milk by withholding these materials from the food of the nursing mother.

Hart, Steenbock and Hoppert (59) find that milking goats fed the fresh green oat plant assimilate calcium better than when fed the same plant in the dried state, and they attribute the better calcium assimilation to a vitamin contained in the fresh material. The connection between milk secretion and calcium assimilation is so important that these experiments will be considered here, although they do not bear directly on the relation between vitamins and milk secretion.

Hart and his collaborators carried out further experiments directed toward determining the nature of the material which is contained in the green oat plant, and which facilitates calcium assimilation. They determined the calcium balance in animals which were fed on a basal ration of dried materials with additions of cabbage, orange juice, butter-fat or cod-liver oil. The cod-liver oil was the only one of these materials which facilitated calcium assimilation. The authors consider the results with cabbage and orange juice sufficiently definite to show that the antiscorbutic vitamin has no favorable influence on calcium assimilation. They do not think that the results with butter fat are conclusive, and they leave open, therefore, the question whether the material which facilitates calcium assimilation is the fat-soluble vitamin or some unknown material contained in cod-liver oil. The question whether absence of the fat-soluble vitamin from the diet may be a cause of the faulty calcium assimilation characteristic of rickets has been studied by a number of investigators with rather varying results (61), (63), (81), (85), (86), (101).

The work on the relation between milk secretion and the vitamin content of the diet, which has just been reviewed, may be summarized by saying that the evidence so far obtained tends to indicate that

changes in the vitamine content of the diet influence directly the concentration of vitamins in the milk rather than the amount of milk secreted. Whatever influence the vitamins of the food may have on milk yield is probably indirect and therefore more or less delayed. The field, however, is too new to justify the drawing of any very positive or detailed conclusions.

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## THE FATE OF FOREIGN ORGANIC COMPOUNDS IN THE ANIMAL BODY

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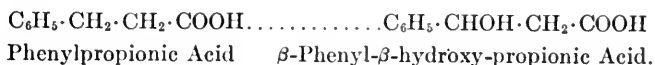
Due to the fact that it is utterly impossible in an article of two or three dozen pages to consider any particular part of so vast a subject in detail, generalities alone will be emphasized. Furthermore, as the references which bear directly on the matter number at present over two thousand with perhaps as many more which are very closely related to this type of work, we have been obliged to limit the bibliography to those references which are the more important. Heffter (37) in his *Ergebnisse* article has covered the literature quite fully up to the year 1904. One is also referred to Frankel's excellent work *Arzneimittelsynthese* (31) particularly for a discussion of the subject from the standpoint of pharmacology.

Foreign organic compounds introduced into the animal body by way of the gastro-intestinal tract or by subcutaneous or intravenous injections can not be considered as foods but must be treated under the collective heading of poisonous or toxic substances. The problem, therefore, is one of toxicology rather than of normal physiology or physiological chemistry, and resolves itself into a study of the "chemical defense mechanism of the animal organism." The animal body, in order to protect itself from these toxic materials, must be able either to destroy them completely or, if this is impossible, to detoxicate them in one way or another and eliminate them in one of the body's excretions; and as the urine is the usual channel of elimination, the solubility of the detoxication products becomes a very important factor.

To attain these ends the body has at its command a series of chemical reactions—reactions, by the way, which we are unable at the present time to duplicate in our best equipped laboratories. The first method of attacking a foreign molecule seems to be an attempt at *complete oxidation*. This, in the majority of cases meets with at least partial success. If the compound be an aliphatic fatty acid its destruction will likely be complete; if it be an aromatic derivative of a long chain acid the side chain will likely be reduced to one or two carbon atoms,

depending, as we shall see later, on the number of carbons composing the original chain. In some cases *reduction* is used as a precursor of oxidation, and in a few instances as an independent reaction. Should the foreign molecule be able to withstand these types of attack, or yield to them at most only partially, it is necessary for the body to have recourse to a synthetic type of reaction, combining the toxic substance with some radical or molecule which it has at its disposal. In this way not only is the toxicity reduced but the resulting product is rendered more soluble. This synthetic type of reaction embraces both the *conjugation* with one of the amino acids such as glycocoll, glutamine or cysteine, or perhaps with sulphuric or glycuronic acids; as well as *methylation*, *acetylation* and *uramino acid formation*.

Knoop (52) noticed that whenever an aromatic derivative of a fatty acid was fed to an animal it appeared in the urine either as benzoic acid or phenylacetic acid in combination with glycocoll, i.e., as hippuric or phenaceturic acids. Further consideration showed that the benzoic acid formers were invariably those containing an odd number of carbon atoms in the chain, such as phenylpropionic or phenylvaleric acids, while those acids like phenylbutyric and phenylcaproic, containing an even number of carbon atoms in the side chain always produced phenylacetic acid. No acid with an odd number of carbon atoms in the chain ever furnished an intermediary product of metabolism with an even number of carbons. Accordingly he concluded that these compounds must be shortened by the splitting off of two carbons at a time, that is to say, the  $\beta$ -carbon was in each case the target for oxidation. This view was strongly supported by the work of Dakin (18) who detected  $\beta$ -phenyl- $\beta$ -hydroxy-propionic acid in the urine after feeding phenylpropionic acid.



It has been pointed out, moreover, by various investigators that the natural fats are composed of fatty acids consisting of an even number of carbon atoms and that these fats in the organism of the diabetic are the precursors of  $\beta$ -hydroxy-butyric acid. Likewise the unsaturated side chain aromatic acids apparently undergo  $\beta$ -oxidation. Thus cinnamic acid,  $\text{C}_6\text{H}_5 \cdot \text{CH} : \text{CH} \cdot \text{COOH}$ , is oxidized to benzoic acid (27) in the animal body to form  $\beta$ -phenyl- $\beta$ -hydroxy-propionic acid (21) as an intermediary product.

Amino acids, particularly aliphatic  $\alpha$ -amino acids,  $R \cdot CHNH_2 \cdot COOH$ , are apparently subject to attack at the  $\alpha$ -carbon position, and either by oxidative or hydrolytic deamination are converted into  $\alpha$ -hydroxy,  $R \cdot CHOH \cdot COOH$ , or  $\alpha$ -keto,  $R \cdot CO \cdot COOH$ , fatty acids. In either case but one carbon atom is split off, and the  $\alpha$ -carbon is oxidized to a carboxyl group. The fatty acid thus formed is then subject to the general rule of  $\beta$ -oxidation.

Alcohols of the aliphatic series are oxidized to the corresponding acids, for example, methyl alcohol to formic acid (80). Primary and secondary alcohols as a rule are easily oxidized in the organism, but tertiary alcohols are apparently burned with great difficulty. Thus ethyl alcohol in small amounts is completely changed to carbon dioxide and water, and isopropyl alcohol,  $(CH_3)_2CHOH$ , is largely converted into acetone  $(CH_3)_2CO$  (5), whereas tertiary alcohols are usually excreted in combination with glycuronic acid, as was found for tertiary amyl alcohol,  $(CH_3)_2COH \cdot CH_2 \cdot CH_3$ , and tertiary butyl alcohol,  $(CH_3)_3COH$ . The halogen substituted alcohols are as a rule excreted in combination with glycuronic acid and seem particularly resistant to oxidation (71). Two well-known cases are trichlorethyl alcohol,  $CCl_3 \cdot CH_2OH$ , (70) and trichlorobutyl alcohol. Aldehydes are seldom if ever found as intermediary products of alcoholic oxidation, but conversely the reduction of aldehydes to alcohols as chloral,  $CCl_3 \cdot CHO$ , to trichlorethyl alcohol,  $CCl_3 \cdot CH_2OH$ , is by no means rare.

The dicarboxylic acids are in general more resistant to oxidation than the fatty acids. Thus oxalic acid,  $(COOH)_2$ , according to some authors is not oxidized at all (66); however, when malonic acid,  $COOH \cdot CH_2 \cdot COOH$ , is fed, relatively small amounts of the unoxidized material appear in the urine, while traces of it are excreted as oxalic acid. Succinic acid,  $(COOH \cdot CH_2)_2$ , and glutaric acid,  $COOH \cdot (CH_2)_3 \cdot COOH$ , are quite easily burned by the organism. It would seem from this that the resistance to oxidation of this series of acids decreases with rise of molecular weight, following much the same rule as that laid down for physiological oxidation of the fatty acids, namely, that the possibility of oxidation varies indirectly with the volatility of the member of the series. Both rules, however, have many exceptions, particularly the one regarding the dicarboxylic acids. Thus adipic acid is much harder to oxidize than any of the lower members of the series.

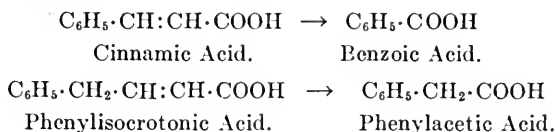
Some of the lower hydroxy, aldehyde and keto acids are important on account of their relationship to the probable intermediary products

of carbohydrate metabolism. Glycollic acid,  $\text{CH}_2\text{OH}\cdot\text{COOH}$ , and glyoxylic acid,  $\text{CHO}\cdot\text{COOH}$ , are both oxidized in a large measure to oxalic acid,  $(\text{COOH})_2$ , (16). Pyruvic acid,  $\text{CH}_3\cdot\text{CO}\cdot\text{COOH}$ , administered in small doses, appears to be completely oxidized, whereas, after subcutaneously injecting into a cat 7 grams of the substance as the sodium salt, (25), there appeared in the urine of the animal glucose, dl-lactic acid and the sodium salt of some unchanged pyruvic acid.

The amides of the higher fatty acids are easily hydrolyzed into ammonia and the corresponding fatty acids, the ammonia fraction going to increase the urea output while the fatty acid is subject to decomposition according to the rule of  $\beta$ -oxidation. The amides of the lower fatty acids, on the contrary, such as acetamide (25),  $\text{CH}_3\cdot\text{CONH}_2$ , appear to be very resistant to oxidation and pass through the organism unchanged. The  $\alpha$ -amino acids found in protein, of the type  $\text{R}\cdot\text{CHNH}_2\cdot\text{COOH}$ , are completely destroyed in the animal body after an initial process of oxidation or hydrolytic deamination, which shows that the amino group is the most sensitive part of the molecule. It is interesting to note that while the optical antipodes of these amino acids are usually treated by the body as non-toxic foreign substances and excreted unchanged in the urine, the dl-forms of some of the lower amino acids are completely oxidized, for example, dl-alanine,  $\text{CH}_3\cdot\text{CHNH}_2\cdot\text{COOH}$  (33).

A number of investigators have substituted one or both of the hydrogen atoms of the amino group of these acids and fed them, with the result that they were found to be very resistant to oxidation. Friedmann noted that when the amino acids found in proteins were monomethylated,  $\text{R}\cdot\text{CH}(\text{NH}\cdot\text{CH}_3)\cdot\text{COOH}$ , the lower members of the series were excreted free to the amount of about one-third of the total that was fed, while the higher members were excreted almost quantitatively. A number of the amino acids have been benzoylated (65),  $\text{R}\cdot\text{CH}(\text{NH}\cdot\text{CO}\cdot\text{C}_6\text{H}_5)\cdot\text{COOH}$ , or phenylacetylated (96),  $\text{R}\cdot\text{CH}(\text{NH}\cdot\text{CO}\cdot\text{CH}_2\cdot\text{C}_6\text{H}_5)\cdot\text{COOH}$ , with the result that they also were excreted in the free state.

The unsaturated aliphatic acids are completely oxidized in the body as is shown in the case of acrylic acid,  $\text{CH}_2\cdot\text{CH}\cdot\text{COOH}$ , (63). The corresponding aromatic acids, however, are oxidized only to benzoic or phenylacetic acids.

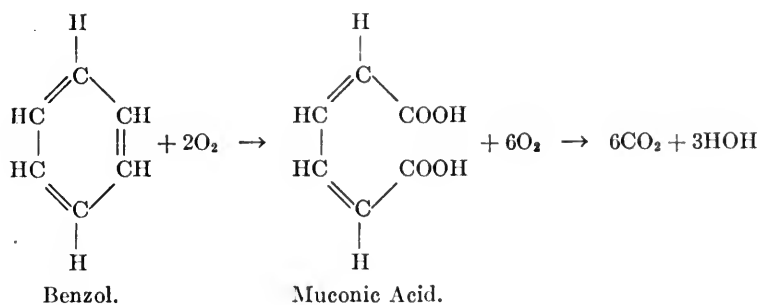


The  $\gamma$ -substituted fatty acids are mostly excreted unchanged, but a few are converted into their lactone forms. Thus phenylbutyric acid,  $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$ , is excreted as its lactone. An interesting exception to this common rule is the case of phenyl- $\gamma$ -keto-butyric acid,  $C_6H_5 \cdot CH_2 \cdot CO \cdot CH_2 \cdot COOH$ . In this case the carbonyl group is apparently reduced and the phenylbutyric acid thus formed is then oxidized to phenylacetic acid and excreted in combination with glycocoll (phenaceturic acid).

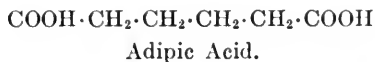
In the oxidation of aromatic substances many complications are met with. In a simple case like the phenyl derivative of a fatty acid there is mere  $\beta$ -oxidation of the side chain, but where there are two or more side chains or substitution products so many new factors appear that these cases are best dealt with separately. In general the benzol ring is very resistant to the oxidizing forces of the body, so much so, in fact, that the breaking of this ring is found in exceedingly few cases. The notable cases are those acids possessing a chain of three carbon atoms, one end of which chain is attached to the benzol ring while the center carbon holds an amino group (6),(7),(13),(91),(92), as phenylalanine,  $C_6H_5 \cdot CH_2 \cdot CHNH_2 \cdot COOH$ , or tyrosine,  $p-OH \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH$  or  $\alpha$ -aminocinnamic acid,  $C_6H_5 \cdot CH : CHNH_2 \cdot COOH$ .

Again, isophthalic acid,  $C_6H_4 \cdot (COOH)_2$  (1-3), and terephthalic acid,  $C_6H_4 \cdot (COOH)_2$  (1-4), are said, to some extent at least, to be completely oxidized in the body (82), while the *o*-compound,  $C_6H_4 \cdot (COOH)_2$  (1-2), phthalic acid itself, is not subject at all to oxidation (81). After the feeding of *o*-nitro-benzaldehyde to dogs Cohn (14) found only about 10 per cent of the material excreted unchanged and believes that the remainder was oxidized in the body. Sherwin and Hynes (95) obtained much the same results after feeding the compound to dogs and rabbits, but when human subjects were employed, 80 per cent of the ingested material was recovered as *o*-nitrobenzoic acid. This negative sort of evidence hardly justifies one in making the assertion that this is another case where the *o*-compound is easily destroyed in the body while the *m*- and *p*-compounds remain unoxidized (32). In this case, as in many others, the *o*-compounds appear to be so much more toxic than the *m*- and *p*-compounds that they must be fed in much smaller doses. Accordingly they may be lost in the attempt to isolate them or may be handled by the body in some as yet undiscovered way and thus escape detection in the urine. The most interesting compound is benzol itself, but there is so much conflicting evidence regarding its fate in the body that the entire question should

be reinvestigated. Jaffé (48) discovered an unsaturated acid in the urine of animals after feeding benzol and was able to isolate muconic acid. On injecting muconic acid into rabbits in amounts as large as 2 grams he was able to recover only 1 per cent of the substance unchanged. From this work one is led to believe that about 25 to 30 per cent of the ingested benzol was split into muconic acid, but the muconic acid being an easily oxidizable substance was further burned to water and carbon dioxide leaving only a trace of the intermediary product of metabolism for excretion. If this be the case the reaction is expressed by the following simple equation:



There is a very close relationship between the saturated and unsaturated acids insofar as their physiological oxidation is concerned. It seems therefore rather improbable that muconic acid should be so easily oxidized when the corresponding saturated acid, adipic acid, is oxidized with so great difficulty.



With this point in view, Mori (73) repeated the work of Jaffé and was unable to substantiate his claims. On the contrary, Mori found that after injecting 0.8 gram muconic acid into rabbits 73 per cent of the substance, on the average, was excreted unchanged, and adipic acid injected in the same quantities, was eliminated as such to the amount of 61 per cent. After administering adipic acid there was always an increase in oxalic acid excretion as a result of the  $\beta$ -oxidation of some of the adipic acid, but not even this evidence of oxidation was noted in the case of muconic acid. Not only does this destroy our most plausible explanation for the oxidation of the aromatic nucleus in the body

but it also excludes the probability of the existence of muconic acid as an intermediary metabolic product of benzol.

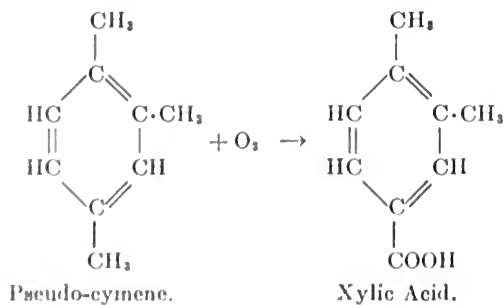
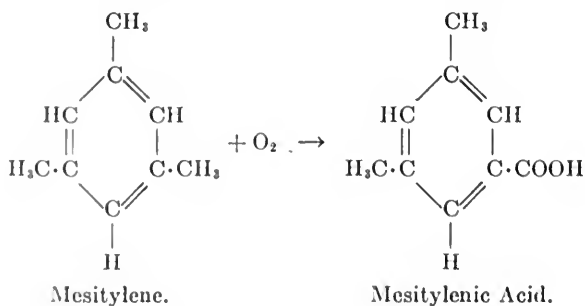
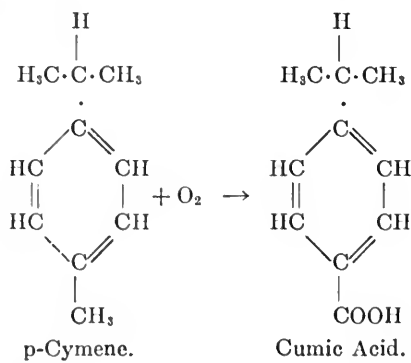
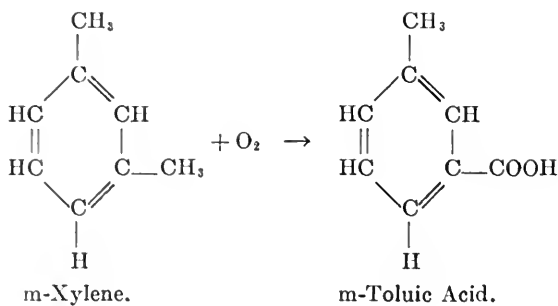
It has been stated above that  $\alpha$ -amino- $\beta$ -phenylpropionic acid (phenylalanine) and some of its derivatives are completely burned in the body. Tyrosine, which is phenylalanine with an -OH group in the p-position, is seemingly burned with as much ease as phenylalanine itself. If, however, the position of the -OH group in the ring be changed, i.e., if o- or m-hydroxyphenylalanine instead of the p-hydroxy compound be fed, there is a very slow and incomplete oxidation, resulting so far as it proceeds, in the formation of o- or m-hydroxyphenylacetic acid (11), (28). M-chlorophenylalanine as well as the p-chlor compound have been found to yield the corresponding phenylacetic acids (34). Dakin (20), however, found that both p-methylphenylalanine,  $\text{CH}_3 \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_2 \cdot \text{CHNH}_2 \cdot \text{COOH}$ , and p-methoxyphenylalanine,  $\text{CH}_3\text{O} \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_2 \cdot \text{CHNH}_2 \cdot \text{COOH}$ , were easily and completely oxidized in the body of patients suffering from alkaptonuria. This, with other instances (12), signifies that a methyl (-CH<sub>3</sub>) group in the benzol ring does not act as a hindrance to the oxidation of the ring. A chlorine atom, however, in any position proves a block to further oxidation rather than a sensitive point at which the ring may be split.

Perhaps the most resistant to oxidation are the phenyl substitution products of the fatty acids. These as we have previously seen are first oxidized to benzoic or phenylacetic acids, then combined in most cases with glycocholic acid to form the corresponding hippuric or phenaceturic acids. Not only is this true of the long chain phenyl derivatives of the fatty acids but it also holds for most of the substitution products of these acids where one or two hydrogen atoms in the ring have been replaced by a nitro group, an amino group, a hydroxy group or one of the halogens.

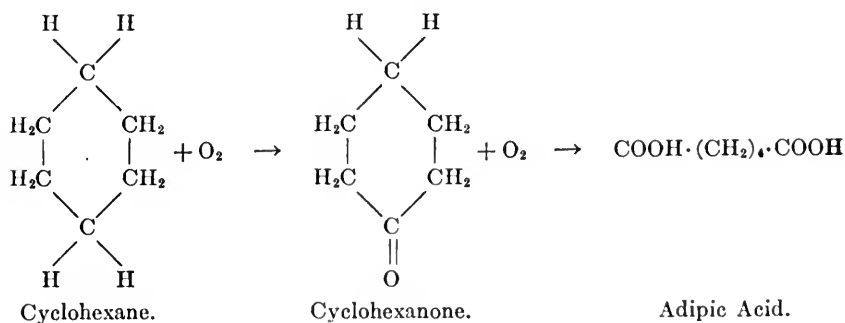
Where there have been substitutions in the side chain of the acid, oxidation of this side chain is either hindered or facilitated, depending first, on the number of carbons in the chain, and secondly, on the positions and kinds of substituted groups. We have previously seen that aromatic acids with three carbon atoms in the side chain, containing also an  $\alpha$ -amino group, for example,  $\alpha$ -amino- $\beta$ -phenylpropionic acid, are completely oxidized in the animal body. This is likewise true of certain  $\alpha$ -hydroxy and  $\alpha$ -keto acids, such as  $\beta$ -phenyllactic acid,  $\text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{CH(OH)} \cdot \text{COOH}$ , and phenylpyruvic acid,  $\text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{COOH}$ . If, however the amino, hydroxy or keto group occupies a  $\beta$ -position,

oxidation is incomplete, for the side chain alone is attacked. Thus  $\beta$ -phenyl- $\beta$ -aminopropionic acid,  $\text{C}_6\text{H}_5 \cdot \text{CHNH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$ , and  $\beta$ -phenyl- $\beta$ -hydroxypropionic acid,  $\text{C}_6\text{H}_5 \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{COOH}$ , are both oxidized only to benzoic acid, while benzoylactic acid,  $\text{C}_6\text{H}_5 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{COOH}$ , (19), is partly excreted as such and partly as acetophenone,  $\text{C}_6\text{H}_5 \cdot \text{CO} \cdot \text{CH}_3$ , together with some  $\beta$ -phenyl- $\beta$ -hydroxypropionic acid,  $\text{C}_6\text{H}_5 \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{COOH}$ , and some cinnamic acid,  $\text{C}_6\text{H}_5 \cdot \text{CH} : \text{CH} \cdot \text{COOH}$ , the latter in combination with glycocholl. This a reduction without  $\beta$ -oxidation, found in the  $\beta$ -amino or  $\beta$ -hydroxy acids. Phenylacetic acid also forms  $\alpha$ -amino,  $\alpha$ -hydroxy and  $\alpha$ -keto compounds. If d-phenylglycine,  $\text{C}_6\text{H}_5 \cdot \text{CHNH}_2 \cdot \text{COOH}$ , is fed, mandelic acid,  $\text{C}_6\text{H}_5 \cdot \text{CHOH} \cdot \text{COOH}$ , is excreted, probably with the formation of phenylglyoxylic acid,  $\text{C}_6\text{H}_5 \cdot \text{CO} \cdot \text{COOH}$ , as an intermediary product (77). When, however, dl-phenylglycine is ingested, there appears in the urine chiefly l-phenylglycine along with dl-acetylaminophenylglycine,  $\text{C}_6\text{H}_5 \cdot \text{CH} \cdot (\text{NH} \cdot \text{CO} \cdot \text{CH}_3) \cdot \text{COOH}$ , l-mandelic acid and glyoxylic acid (78). The three latter substances are degradation products of the d-phenylglycine. A small fraction of both the d- and the l-compounds is oxidized to benzoic acid. Phenylglyoxylic acid when fed to dogs or human beings is reduced to l-mandelic acid. From the literature it is difficult to decide whether the l-mandelic acid alone is formed or whether both the d- and the l-forms are produced but with different pathways of elimination. Schempp (89) found only dl-mandelic acid in the urine of men and dogs after feeding phenylbromacetic acid,  $(\text{C}_6\text{H}_5 \cdot \text{CHBr} \cdot \text{COOH})$ . Since simple hydrolysis of this compound, however, takes place even in moist air, Schempp's finding is of little physiological importance. The separation of dl-substances into their active components with subsequent oxidation of one and total elimination of the other has been noted by various experimenters. Thus dl-p-hydroxyphenylglyoxylic acid, 4-OH  $\cdot$   $\text{C}_6\text{H}_4 \cdot \text{CHOH} \cdot \text{COOH}$ , when fed to rabbits (24), is split into its optical antipodes followed by oxidation of the l-component and excretion of the d-form.

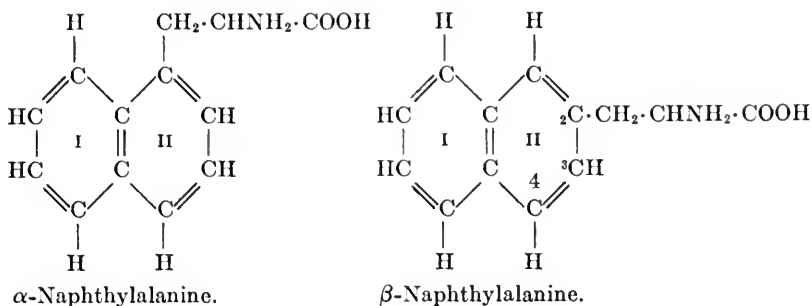
Where more than one side chain is attached to the benzol ring, oxidation is usually confined to but one of these chains (74), (75), (76), (93). A few of the best known cases are those of m-xylene, p-cymene, mesitylene and pseudo-cymene. All of these are oxidized on one side chain to the corresponding acids:



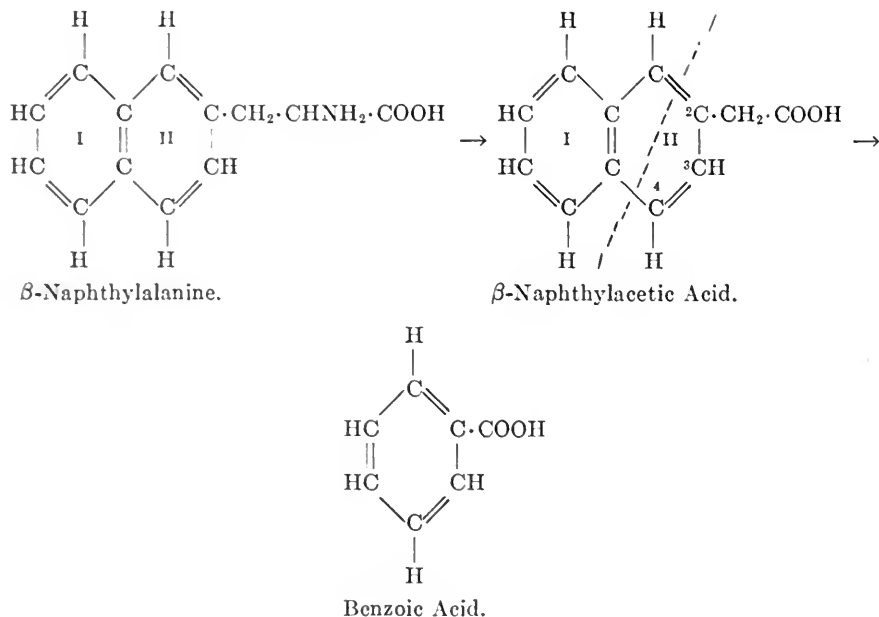
Cyclohexane is oxidized in a large measure to cyclohexanone and small amounts even further with ring splitting to adipic acid:



The naphthalene nucleus is also partially destroyed in the organism as was demonstrated by the interesting experiment of Kikkoji (51). He wished to compare the fate in the body of a double ring compound containing a side chain of three carbon atoms holding also an  $\alpha$ -amino or  $\alpha$ -keto group, with that of phenylalanine. He prepared two different compounds,  $\alpha$ -naphthylalanine and  $\beta$ -naphthylalanine and fed them to dogs.

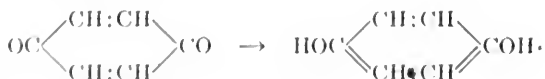


From the urine, after feeding the  $\alpha$ -compound, he was able to isolate an unknown derivative of naphthylene which apparently had both rings intact. Nothing could be found that indicated that either ring had been split. After feeding  $\beta$ -naphthylalanine, however, he found first that the side chain had been deaminized and shortened to two carbon atoms, then that a second reaction had split ring II with the formation of benzoic acid which was excreted as hippuric acid. The reaction is seemingly the one pictured below:



He concludes from this that  $\alpha$ -amino acids are shortened by one carbon at a time and that the splitting of the ring occurs between carbon atoms 1 and 2 instead of between 3 and 4; else the resulting compound would have been phenylbutyric acid,  $\text{C}_6\text{H}_5 \cdot (\text{CH}_2)_3 \cdot \text{COOH}$ , which in turn would have been oxidized to phenylacetic acid,  $\text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{COOH}$ , and have been found in the urine as phenaceturic acid.

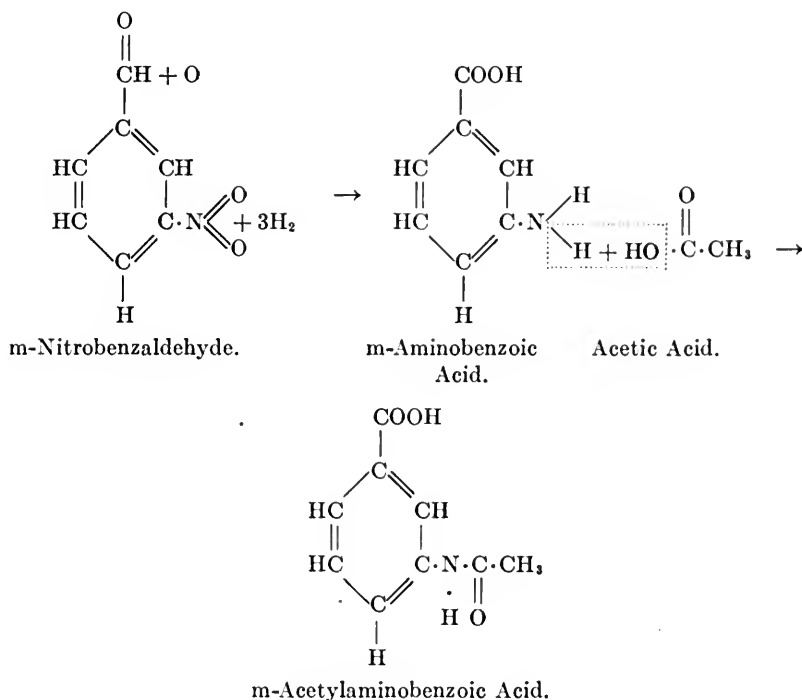
**REDUCTION.** Reduction in the animal body, while not so common as oxidation, is by no means of rare occurrence. One of the first cases found in the literature is that of the reduction of chloral,  $\text{CCl}_3 \cdot \text{CHO}$ , to trichlorethyl alcohol,  $\text{CCl}_3 \cdot \text{CH}_2\text{OH}$ , (58), (59), (70), (71),—a reduction, it may be remarked, which is brought about in the laboratory only with difficulty. Other reductions have since been found, such as that of quinone to hydroquinone:



Unsaturated acids are changed in the organism into saturated compounds, carbonyl ( $-\text{CO}$ ) groups into secondary alcohols ( $-\text{CHOH}$ ), and both may even be reduced to methyl groups ( $-\text{CH}_3$ ), (56) (100).

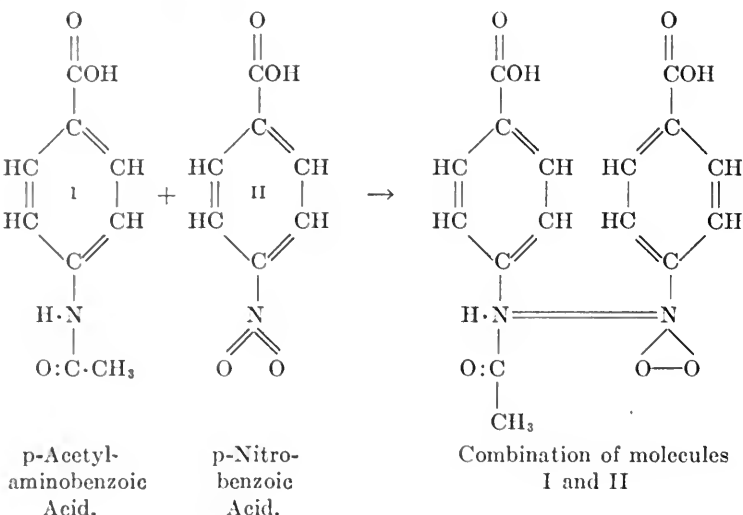
After feeding *o*-nitrotoluene, Jaffé (43), (46) found that the compound had been oxidized partly to *o*-nitrobenzyl alcohol and partly to *o*-nitrobenzoic acid. In this case there was very probably an oxidation to the aldehyde with a simultaneous oxidation and reduction analogous to the Cannizzaro reaction,  $R \cdot CHO + R \cdot CHO \rightarrow R \cdot CH_2OH + R \cdot COOH$ .

There is in the literature a number of cases where oxidation and reduction occur simultaneously within the same molecule. A remarkable case of this kind was noted by Cohn (14) after feeding *m*-nitrobenzaldehyde to rabbits:



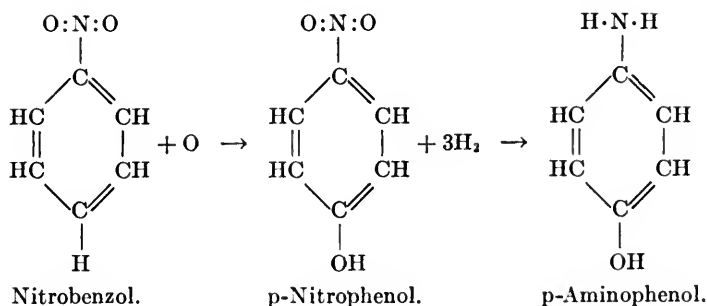
Here we see the simultaneous oxidation of an aldehyde ( $-CHO$ ) group to a carboxyl ( $-COOH$ ) and the reduction of a nitro group to an amino. As a secondary reaction we have the interaction between the *m*-aminobenzoic acid thus formed and an acetic acid molecule resulting in the formation of an acetyl amino compound. After feeding the *p*-nitrobenzaldehyde to rabbits there is formed in an analogous manner, *p*-acetylaminobenzoic acid from a part of the substance, while another

part is merely oxidized on the aldehyde group to p-nitrobenzoic acid. Then to complicate matters still more there is a combination of these two molecules through their nitrogen atoms (84).

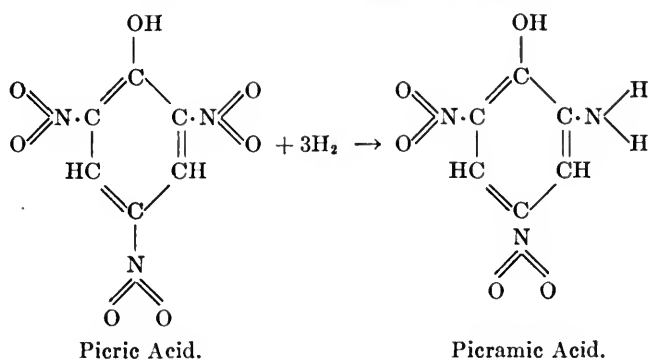


In contrast to the above mentioned nitrobenzaldehydes the o-nitrobenzaldehyde is excreted as o-nitrobenzoic acid only to about 10 per cent of the amount fed. The remainder has thus far escaped detection. It is interesting to note here that the nitrobenzaldehydes act much differently in the organisms of the dog (99) and the human being (95). The o-nitro compound, when fed to dogs, also disappears in amounts as great as 90 per cent, while from the human organism it has been recovered in the form of o-nitrobenzoic acid in relatively large amounts. Both the m-nitro and the p-nitro benzaldehydes, instead of being reduced, in either case are oxidized to the corresponding acids and excreted to a large extent in combination with glycocoll, i.e., as m-nitro and p-nitro hippuric acids. Again, p-nitrophenylacetaldehyde, when fed to a human being, a rabbit and a dog, was merely oxidized to p-nitrophenylacetic acid and eliminated in the urine without undergoing either reduction or conjugation.

The simultaneous reduction of a nitro group and the oxidation of some other group within the same molecule is not confined to the nitro aromatic aldehydes, but appears also in the case of nitrobenzol. This substance is first oxidized in the p-position to form p-nitrophenol. The nitro group is then reduced to an amino group yielding p-aminophenol as an end product (72).



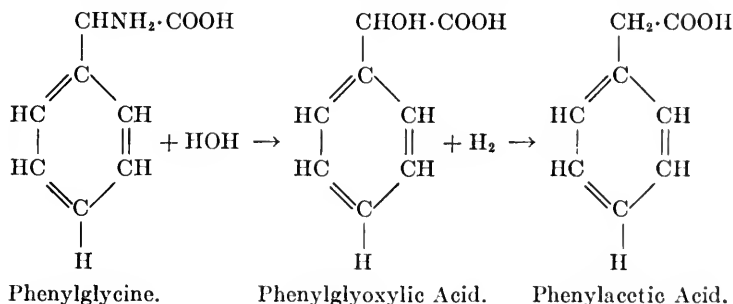
If, however, m-nitrophenol (8) is fed to rabbits, only a portion of it is reduced to m-aminophenol. Much more striking, however, is the case of o-nitrophenol which is excreted by rabbits unchanged. Picric acid (2,4,6-trinitrophenol), according to some authors (85) is excreted merely combined with sulphuric acid, while according to others (4), a reduction takes place and the substance appears as picramic acid:



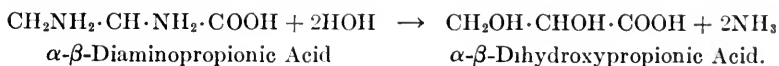
Perhaps the most remarkable case of reduction ever observed was that noted by Hoppe-Seyler (40), who fed to rabbits o-nitrophenyl-propionic acid and found in the urine a substance identical with urinary indican, i.e., as a compound of indoxyl with either potassium hydrogen sulphate or glycuronic acid. The reaction is apparently that represented below, namely, a reduction of the nitro to an amino group and also a reduction in the side chain with the formation of o-aminoacrylic acid, then a closing of the ring resulting in the formation of indol. This compound thereupon loses carbon dioxide and is oxidized to indoxyl, which is then conjugated with potassium hydrogen sulphate and excreted as indican.



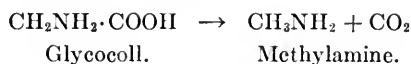
We saw previously that an amino group in the side chain may be removed by hydrolysis with or without subsequent reduction of the chain, for both phenylglyoxylic and phenylacetic acids were found in the urine after feeding phenylglycine (phenylaminoacetic acid).



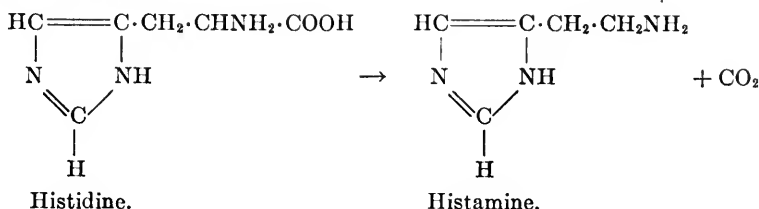
The hydrolysis of the amino group, however, is not confined to those cases in which the amino group occupies the  $\alpha$ -position, as is evidenced by the fact that  $\alpha$ - $\beta$ -diaminopropionic acid is converted into the corresponding dihydroxy acid (68):

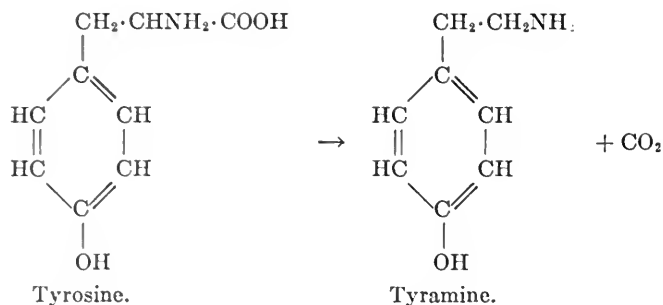


After the action of putrefactive bacteria on proteins and protein decomposition products in the gastro-intestinal tract, the  $\alpha$ -amino acids may be decarboxylated, i.e., attacked in such a way that they lose carbon dioxide from the carboxyl ( $-\text{COOH}$ ) group, and thus be converted into the corresponding amine:

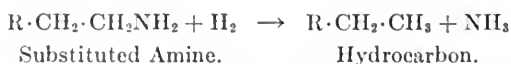
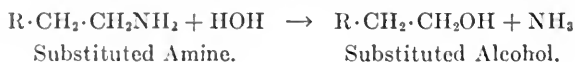


Among these amines are substances which exert a decided effect upon blood pressure, such as histamine and tyramine, and are therefore considered as very toxic substances. They may perhaps even be the cause of arterio-sclerosis and subsequently one of the prime factors in producing old age.

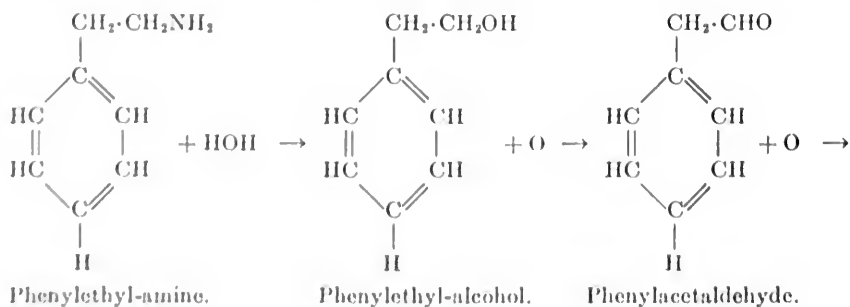


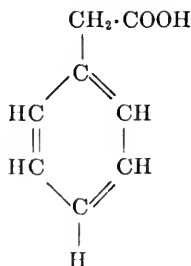


The work of Guggenheim and Loeffler (36) has enlightened us somewhat on this point, demonstrating that these substances may be taken in relatively large doses, 0.25 gram or more, without decided physiological effects. The investigators were able to show at the same time that these toxic amines are rapidly detoxicated in the animal body by a simple cleavage of the amino group, perhaps by hydrolysis, yielding the corresponding alcohol, or by reduction yielding the hydrocarbon.



In some cases alcohols were isolated after feeding the amines, showing the actual formation of the alcohols as intermediary products, but in most cases the oxidation proceeded further, yielding a corresponding acid. Thus phenylethylamine gives phenylacetic acid, probably passing through the intermediary metabolic forms of phenylethyl alcohol and phenylacetaldehyde, for both of these compounds yield phenylacetic acid when fed by themselves, although phenylethane,  $\text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{CH}_3$ , is oxidized to benzoic acid.





Phenylacetic acid.

Similarly it was found that isovaleric acid was formed from amylamine, p-hydroxyphenylacetic acid from p-hydroxyphenylethylamine (tyramine), and indolacetic acid from indolethylamine. Histamine very likely undergoes a like change but no  $\beta$ -imidazoleacetic acid was identified in the urine.

**SYNTHETIC REACTIONS.** In a few cases foreign organic compounds enter the animal body and are rapidly excreted without undergoing chemical change. These cases, however, are relatively few and it will be found invariably that the substance is non-toxic and quite soluble. If the foreign molecule cannot be destroyed entirely or even partially and still retains after partial demolition some of its toxic properties, the only recourse left to the body is a synthetic type of reaction. This it accomplishes by attaching to the foreign substance an amino or methyl group or by conjugating it with a second molecule such as glycocoll, glutamine or ornithin. In this way not only is toxicity reduced or destroyed, but solubility is simultaneously increased, enabling the body to excrete the product with ease and rapidity. There are cases, too, where a given substance is conjugated partly with one compound and partly with another. This is true, for example, of phenol which, after ingestion, is excreted not only as a sulphuric acid conjugate but also as a glycuronate and even to some extent in the free state. This is perhaps the foundation of the erroneous opinion often expressed in textbooks that conjugation is effected with one of these detoxicating agents, such as glycocoll, for the purpose of protecting the foreign substance against further oxidation, for otherwise the intruder might be excreted uncombined. Upon examining a case of this kind one finds, for example, that after a certain compound is ingested 65 to 75 per cent of it has been excreted in combination, let us say, with glycuronic acid, while the remainder has apparently disappeared. Thereupon the inference is drawn that the 25 to 35 per cent of the substance

unaccounted for has been oxidized in the organism and that only by conjugation has the other part been saved from this fate. The truth of the matter is, however, that the body has conjugated this foreign molecule with glycuronic acid only after a futile attempt to oxidize it, and that the 25 to 35 per cent had escaped detection either because of some other channel of elimination or on account of a different form of conjugation. In fact, the recovery of 65 to 75 per cent of many of these substances may be considered "almost quantitative."

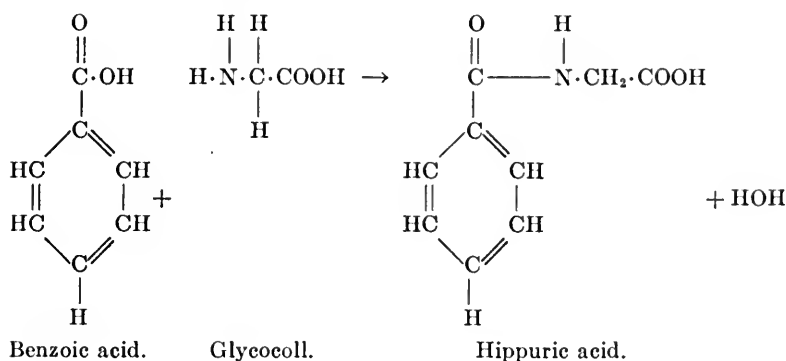
Dakin (17) has shown experimentally that phenylpropionic acid,  $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$ , cinnamic acid,  $C_6H_5 \cdot CH \cdot CH \cdot COOH$ , and  $\beta$ -phenyl- $\beta$ -hydroxypropionic acid,  $C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$ , which are more or less poisonous in themselves, are rendered non-toxic when combined with glycocoll. Phenylpropionic acid when administered to cats in doses so small as 0.8 gram per kilo of body weight caused death in 40 to 60 hours, while phenylpropionylglycocoll in doses as large as 1.5 grams per kilo body weight proved entirely non-toxic.

Berezeller (10) has recently suggested an explanation for certain conjugation products from a physico-chemical point of view. He believes that the compounds which are susceptible of conjugation in this way are those which increase the surface tension of the liquid in which they are dissolved, while the conjugation product has less if any effect of this kind. Benzoic acid lowered the surface tension of the solution very decidedly, while hippuric acid produced no such effect. Menthol likewise caused a marked depression of the surface tension. Its detoxication product, menthol-glycuronic acid, however, had much less effect, and the salt of the glycuronic acid compound still less. Phenol exerted the same influence as menthol, while phenol-sulphuric acid and the two oxidation products of phenol, namely, pyrocatechol and hydroquinone, acted like menthol-glycuronic acid and the salt of the latter, respectively. It is interesting to note that glycocoll itself was found to be inactive in this regard. The observations of Berezeller are instructive and no doubt valuable, but serious complications arise when we reflect that a given compound fed to half a dozen different animals is detoxicated in as many different ways.

In general it would seem that a compound incapable of complete oxidation is usually converted into an alcohol or an acid. In the former case there are two main possibilities of detoxication, namely, in the formation of an ethereal sulphate or of a glycuronate. In the case of the acid, conjugation is usually effected with one of the amino-acids, such as glycocoll, glutamine or ornithin. Reduction is generally

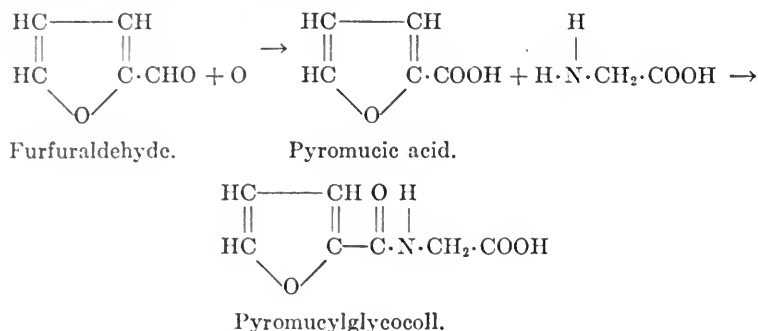
met with in connection with nitro compounds which are thus converted into amines and subsequently often acetylated. As it is impossible to consider the behavior of the individual toxic substances it would seem most logical to take up a short discussion of the several detoxicating agents furnished by the body.

**GLYCOCOLL.** We have previously seen that aromatic derivatives of the fatty acids containing an odd number of carbon atoms in the side chain are oxidized to benzoic acid while those with an even number of carbons are converted into phenylacetic acid. Benzoic acid is paired with glycocoll by the human being, by the horse, cow, dog, cat, and in fact by every vertebrate thus far used for experimentation with the exception of the fowl (61), (106).



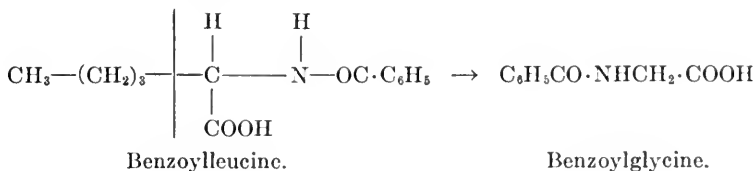
Not only is benzoic acid itself combined in this way with glycocoll but most of the derivatives of benzoic acid such as the o- and m-chlor, the m-brom, the o-, m- and p-flour, and the m- and p-nitro compounds as well as the three hydroxy benzoic acids are subjected to a similar fate. Phenylacetic acid is treated in a like manner in the organisms of the dog (87), (88) and cat (89) but not so in that of man or fowl. The same holds for certain derivatives of phenylacetic acid, namely for those compounds resulting from substitutions in the nucleus (30) such as the p-chlor, p-brom and p-iodo compounds. When the substitutions are made in the side chain, however, as in the case of phenylaminoacetic acid, mandelic acid or phenylglycollic acid no conjugation takes place.  $\beta$ -Naphthoic acid, a double ring structure, also combines with glycocoll to some extent (15). A great variety of acids combine in this way with aminoacetic acid. Two rather unusual examples are those of furfural and thiophenealdehyde. As will be

seen below, furfural (furfuraldehyde) (49) is first oxidized to pyromucic acid and this in turn combined with glycocoll. A similar reaction is undergone by thiophenealdehyde (14).



The discovery of this means of detoxication about 1825 attributed to Wöhler and noted first in the case of benzoic acid was probably the earliest proof that syntheses of this type could take place in the animal body. For many years thereafter it was thought that the organism merely made use of supplies of glycocoll which it was holding, as it were, in reserve, and that this ready material was simply joined on to benzoic acid by a "dehydration reaction." When it was found, however, that glycocoll could not be stored in the body and that the organism could still provide it, and this in relatively large amounts, even when the glycocoll intake was cut off as in the case of an animal a non-protein diet or during a fast, it then became evident that the body must prepare this compound when required for detoxication purposes. Ringer found that a goat might take 25 grams of benzoic acid per day and excrete it as hippuric acid (83). Magnus-Levy (64) noted that 27.8 per cent of the urinary nitrogen excreted by sheep and rabbits after ingesting benzoic acid might appear in the form of hippuric acid nitrogen; Wiechowski (105) observed that this might run as high as 50 per cent. Ringer expressed the belief that under conditions of benzoic acid feeding the glycocoll is formed from "extra destroyed protein." He is inclined to think, however, that it arises by a specific and peculiar catabolic process rather than from a product of normal intermediary metabolism. Epstein and Bookman (26) conclude from their work on rabbits that benzoic acid exerts a truly toxic effect upon the organism but acting in a certain selective way causes the elimination of much larger amounts of nitrogen than are ordinarily excreted, which nitrogen can be almost entirely accounted for as hippuric acid nitrogen.

It has been suggested by many writers that hippuric acid might arise from a secondary reaction following the primary combination of benzoic acid with one of the long chain amino acids such as leucine, and that this benzoylleucine might then undergo a cleavage resulting in the formation of a glycine rest attached to the benzoyl radical:



In order to test this hypothesis, Magnus-Levy (65) benzoylated ten of the amino acids, namely, alanine, serine, valine, leucine, phenylalanine, aspartic acid, glutamic acid and ornithin. He then injected them subcutaneously using a dog as the experimental animal. In each case he recovered the original substance from the urine and this in such large amounts that there was no ground to believe that even a fraction of any of the compounds had been split in the animal organism. The work was to some extent repeated by Shiple and Sherwin (96) who prepared phenylacetyl compounds of alanine, leucine, glutamine, asparagine, glutamic acid, aspartic acid and ornithin and fed them to both men and animals. The results, however, were much the same as those of the former investigator.

Knoop (54) suggests the possible formation of glycocoll from the oxidation of  $\alpha$ -amino- $\beta$ -hydroxy acids,  $\text{R}\cdot\text{CHOH}\cdot\text{CHNH}_2\cdot\text{COOH}$ , inasmuch as phenylserine,  $\text{C}_6\text{H}_5\cdot\text{CHOH}\cdot\text{CHNH}_2\cdot\text{COOH}$ , is incompletely oxidized in the organism and yields hippuric acid. Other experimental evidence, however, is lacking. We must agree, therefore, with Lusk (62) that "these experiments are a further demonstration that in the breaking down of amino acids deamination is the first step, and they leave no conclusion open other than that *glycocoll arises by a synthetic process.*"

McCollum and Hoagland (69) have shown by a series of remarkable experiments that when a pig was reduced to a condition of minimal nitrogen metabolism by a carbohydrate diet (75 cal. per kilo) and at the same time was fed increasingly large doses of benzoic acid, as long as the dose did not exceed 0.2 gram per kilo there was no increase in protein catabolism as evidenced by the constancy in the daily total nitrogen output. Furthermore, there was but little alteration in the

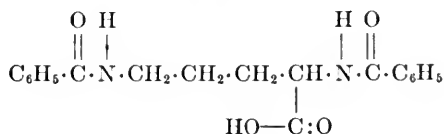
various nitrogenous constituents of the urine with the exception of urea which, very interesting to note, could be reduced from 56 per cent of the total nitrogen to 19 per cent. When amounts of benzoic acid larger than 0.2 gram per kilo were fed there was a decided increase in the output of total nitrogen, much in excess of the amount required for hippuric acid. This shows no more than that 37 per cent of the total nitrogen which ordinarily goes to form urea may under these conditions be side-tracked to form glycocoll. It tells us nothing, however, concerning the mechanism of the reaction. The work has been corroborated by Lewis (60) who fed benzoic acid to human beings maintained on a low protein diet, and by Shiple and Sherwin (98) who reduced a human being to a level of endogenous protein metabolism and were able to show that glycocoll could be formed at the expense of the urea nitrogen without increasing the amount of total nitrogen excreted.

Various attempts have been made to explain the formation of glycocoll by the interaction of ammonia with acetaldehyde,  $\text{CH}_3\cdot\text{CHO}$ , glyoxal,  $\text{CHO}\cdot\text{CHO}$ , or glycolaldehyde,  $\text{CH}_2\text{OH}\cdot\text{CHO}$ , all of which may be considered as decomposition products of carbohydrates. So far, however, the results have been unsatisfactory. Delprat and Whipple in a very recent contribution (22) showed that after injecting benzoic acid into the circulation of fasting dogs there was a definite rise in urea, ammonia, and total nitrogen, provided a certain dosage had not been exceeded. This was certainly to be expected since the demand for glycocoll was extremely acute and the toxicity of the benzoic acid, therefore, increased many times. Moreover, it is impossible to compare the results on fasting animals with those obtained when the animals have been supplied with sufficient food calories in the form of carbohydrates.

From this one hundred years of experimental work we have learned that glycocoll can be built in relatively large amounts by the body even under practically any pathological condition. Moreover, the synthesis proceeds as well during a fast or under abnormal diet as when the ordinary food has been ingested. These results may be far reaching and important since several proteins and particularly the proteins of milk are entirely lacking in this amino acid.

**ORNITHIN.** Previous work has shown that most of the animals commonly used in experiments supply glycocoll for the detoxication of many of the aromatic acids such as benzoic and phenylacetic acids as well as various of their derivatives. Jaffé (44) however, after feeding benzoic acid to hens was unable to isolate hippuric acid from the

excreta, but obtained instead a rather unstable compound which he called ornithuric acid. This substance, later found to consist of ornithin,  $\text{CH}_2\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CHNH}_2 \cdot \text{COOH}$ , combined with two molecules of benzoic acid has the following structure:



It has since been found that phenylacetic acid (103), as well as p-nitrophenylacetic acid and pyromucic acid (94), (50), combine with ornithin to form compounds analogous to ornithuric acid. In fact no case has yet been observed in which glycocoll is employed in the organism of the bird for conjugation purposes. It is quite reasonable to conclude, therefore, either that birds have no glycocoll at their disposal or, if they have, that they are not able to conjugate it with the various compounds that have been fed. To test this assumption Yoshikawa (107) fed benzoic acid to hens and at the same time added glycocoll to the diet in more than sufficient amounts for combination. No glycocoll compound, however, was found in the excreta. Only the ornithin conjugate appeared, the glycocoll apparently having been burned in the organism. Furthermore, present evidence is lacking both of the formation of glyceuronates and sulphates as well as of acetylation. The only synthetic process open to the fowl, therefore, for detoxication of foreign organic compounds, be they acids or alcohols, is so far as we know that accomplished by means of ornithin.

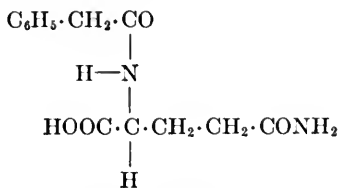
Ornithin is one of the components of the  $\alpha$ -amino acid arginine,  $\text{H}_2\text{N} \cdot \text{CNH} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CHNH}_2 \cdot \text{COOH}$ . It seems very probable, therefore, that it is formed from arginine when for example birds are fed benzoic acid. Under normal conditions, however, the arginine and consequently the ornithin would be completely destroyed during the processes of metabolism. If this be true, it gives us an important bit of information regarding the intermediary metabolism of arginine, though it also confronts us with a very important question as to the possibilities of the synthesis of this amino acid in the body of the fowl. The only evidence we have on this latter point is that furnished by Thomas (102) who administered benzoic acid to birds maintained on a non-protein diet but was unable to find ornithuric acid in the urine. He found small amounts after adding to the diet edestin which contains

large amounts of arginine. It would seem therefore, that arginine and consequently ornithin is not synthesized in the organism of the fowl at the expense of any of the nitrogenous urinary constituents. Since uric acid is the only substance that is present in sufficient amount to furnish the necessary nitrogen, it would be interesting to determine whether under well controlled conditions of experimentation such a transformation is possible. Separation of the urine and feces, however, and the quantitative determination of the urinary constituents and other untold hardships connected with this problem makes such an investigation well-nigh impossible.

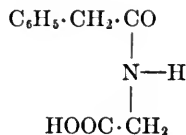
GLUTAMINE. Benzoic acid in the organism of various experimental animals as well as that of man is conjugated with glycocholl. It is rather extraordinary, therefore, to say the least, that phenylacetic acid, the homologue of benzoic, should combine in the animal body with glycocholl to form phenaceturic acid, but in the case of the human being with glutamine,  $\text{COOH} \cdot \text{CHNH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CONH}_2$ , to form phenylacetylglutamine, together with some phenylacetylglutamine urea, the latter apparently a simple addition product with urea (101). Still more remarkable, however, is the fact that o-nitrophenylacetic, o-amino-phenylacetic, p-nitrophenylacetic, p-aminophenylacetic and p-hydroxy-phenylacetic acids pass through the human body absolutely unconjugated. The p-chlor compound is the only one of these derivatives so far examined that probably combines with glutamine. None of these substances combine with glycocholl in the human body, while only the p-chlor (42), p-hydroxy and p-nitro phenylacetic acids have thus far been found to combine with that substance in the organism of any of the lower animals. All the others so far examined pass through uncombined except o-aminophenylacetic acid which is acetylated by the rabbit.

It was at first thought that the phenylacetic molecule was first combined with glutamic acid which product was secondarily changed into glutamine by the addition of the amide group. The feeding of phenylacetylglutamic acid, however, resulted in its excretion without the formation of phenylacetylglutamine. We have here another case of an *amino acid*, *glutamine*, which it is possible to withdraw intact from the protoplasm of the living cell without its conversion into a secondary or derived product. This furnishes the proof which for a long time was wanting that glutamine rather than glutamic acid must be considered the true form of this -amino acid. Moreover, it explains the genesis of most of the ammonia which always appears when proteins are hydrolyzed in acid media.

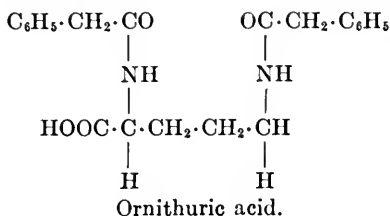
Glutamine like glycocoll can be furnished in fairly large amounts for the detoxication of phenylacetic acid (97), and like glycocoll it may be synthesized at the expense of the urea nitrogen (98). A man was placed on a suitable strictly carbohydrate diet and thus reduced to a condition of endogenous protein metabolism. He then ingested on alternate days benzoic and phenylacetic acids. The synthesis of glycocoll and glutamine was accomplished at the expense of the urea nitrogen apparently with great ease in each case. When benzoic and phenylacetic acids were fed together, necessitating, therefore, the simultaneous preparation of glycocoll and glutamine, each aromatic acid was detoxicated and excreted at much the same rate as if it alone had been ingested. It was possible to lower the urea nitrogen from about 60 per cent of the total 24-hour output of nitrogen to about 18 to 20 per cent of the total. During a fraction of a day, however, after 10 grams of phenylacetic acid had been taken, this urea nitrogen was reduced to 12 per cent of the total nitrogen for that period. It was hoped for a time that a solution might be found in these results as to the source of glycocoll in the animal body. For it was just possible that glutamine which is joined with phenylacetic acid and excreted as phenylacetylglutamine by human beings, might in the organisms of the lower animals be an intermediary product which would be subject to further oxidation and appear, for example, as phenylacetylglucocoll. Accordingly phenylacetylglutamine was prepared and fed to dogs, cats and rabbits, but in each case it was excreted without undergoing any transformation into phenaceturic acid. It was also fed to chickens to see what influence it might have on ornithine formation, but again it was excreted without alteration of any kind.



Phenylacetylglutamine.



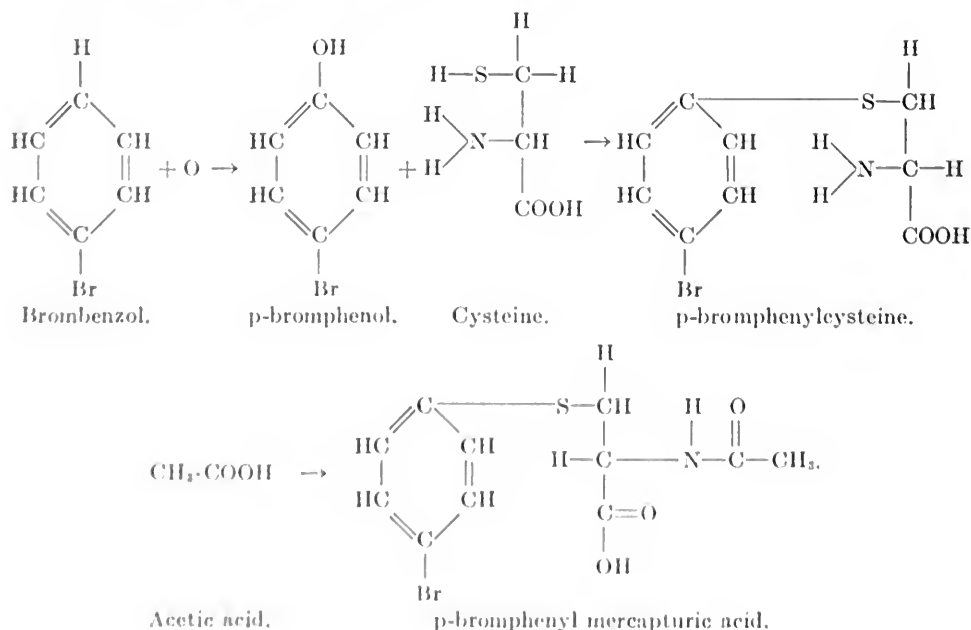
Phenaceturic acid.



Ornithuric acid.

Similarly phenaceturic acid when fed to human beings failed to undergo synthesis into phenylacetylglutamine. Nor was it altered when fed to chickens, but was simply excreted in each case as the sodium salt of the acid. Likewise ornithuric acid when fed to men and dogs failed to form the phenylacetic acid detoxication products common to these subjects. The results of work with other phenylacetylated amino acids, namely, alanine, leucine, asparagine, aspartic acid and glutamic acid were all negative, for when these compounds were fed to various animals they were always recovered from the urine unchanged. It seems most probable, therefore, that neither benzoylated nor phenylacetylated amino acids are open to alteration of any kind in the animal body.

**CYSTEINE.** The fourth amino acid to be used by the body for detoxication work is cysteine,  $\text{CH}_2\text{SH} \cdot \text{CHNH}_2 \cdot \text{COOH}$ . It was found both by Jaffé (45) and by Baumann and Preusse (9) that brombenzol, chlorbenzol and iodobenzol, when fed to dogs, is excreted in the urine as a sulphur-containing compound known as a mercapturic acid. Analysis of the substance showed it to be a combination of the benzol halide with an acetylated cysteine molecule. This whole compound, then, more than likely, conjugates with glycuronic acid. The reaction which takes place is probably the following:



The first step in the reaction is the oxidation of brombenzol in the para position to p-bromphenol, which then combines through its -OH group with the -SH of cysteine to form p-bromphenyl cysteine. This compound is then joined through its amino group with a molecule of acetic acid, resulting in the synthesis of  $\alpha$ -aminoacetyl  $\beta$ -thio p-bromphenyl propionic acid, i.e., p-bromphenyl mercapturic acid. Only in the case of dogs has the formation of this compound been established. The partition of sulphur in the urine of human beings and rabbits, however, after feeding the mono-halogen benzol derivatives, seems to indicate the presence of the mercapturic acid, though none has so far been isolated. The chlor- and brom-naphthalenes form a corresponding mercapturic acid to some extent. The p-brom- and p-chlor- phenols, however, strange to say, are excreted in combination with sulphuric acid (58). As cysteine is quite easily oxidized to cystine it is still questionable whether cystin or cysteine is the form in which this sulphur containing amino acid exists in the protein molecule. It is not at all improbable that both forms exist there. If cystine be the primary form, as is generally accepted, then we have the proof in the above mentioned results that cysteine is at any rate a very active intermediary product in body metabolism.

Upon a second consideration of the equations represented above several very interesting questions arise.

1. Is it possible for cysteine to be joined in the original protein molecule through groups other than a similar -SH radical?

2. Is it only in the case of the dog that the mercapturic acid is thus protected against the oxidation of its sulphur, or may this compound be a common intermediary product of metabolism which in other animals is further oxidized and excreted as p-brom-phenol-sulphuric acid?

3. What is the significance of the acetyl radical here? Does it hinder or help further oxidation of the cysteine, or is its sole purpose to increase solubility and decrease the toxicity of the compound?

4. Is it possible for the animal body actually to synthesize cysteine for this purpose, or is the organism limited to that which it derives from the food and from decomposition of body tissue?

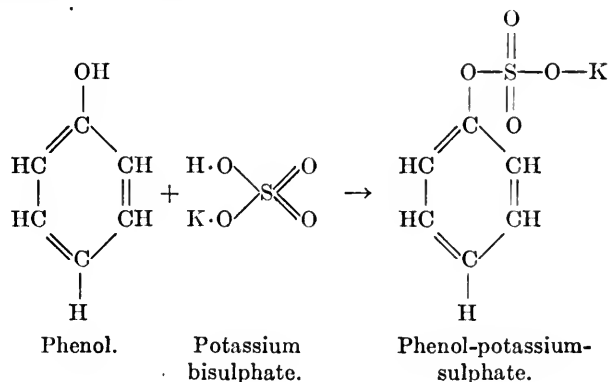
At the present time there is insufficient experimental evidence to answer any of these questions except the last. Kopffhammer (57) in studying this problem fed dogs with brombenzol after the animals had been reduced to a condition of minimal nitrogen metabolism. He found under these conditions that the dogs excreted no p-bromphenyl-

mercapturic acid. Apparently, therefore, they were unable to form it on a non-protein diet. He again fed brombenzol and injected the dogs subcutaneously with a solution of the sodium salt of cystine at the ratio of 4 grams cystine for each gram of brombenzol fed. Thereupon the animals were able to synthesize bromphenylmercapturic acid in quantities equal to the amount obtained after a heavy protein diet containing much cystine. There is no doubt, therefore, that it is an impossibility for the animal body to build cysteine and subsequently cystine when both sulphur and nitrogen are lacking. It would still be interesting to ascertain whether these amino acids could be built at the expense of the urea nitrogen when sulphur in suitable form is supplied.

**ETHEREAL SULPHATES.** It was previously stated that when a foreign organic body cannot be destroyed through complete oxidation there is still a tendency to oxidize it as far as possible and either to convert it into an acid that it might be combined with one of the amino acids, or to reduce or oxidize it, as the case may be, into a hydroxy compound or alcohol. In this latter condition, the substance is open to combination with either sulphuric or glycuronic acid. Though there are certain compounds which combine only with sulphuric acid or only with glycuronic acid, still, in general, the substance seems able to combine with either. Apparently the first tendency is to conjugate with the available sulphuric acid. Since this supply, however, is rather limited, combination is next effected with glycuronic acid.

According to our present limited knowledge of the physiological chemistry of sulphur, this element may occur in the urine in three different forms, namely, neutral or reduced sulphur and the two kinds of oxidized sulphur, i.e., inorganic sulphates and ethereal sulphates. Folin (29) has pointed out that the amount of inorganic sulphates in the urine usually runs parallel with the urea output and may therefore, well be considered as resulting from exogenous metabolism, i.e., from the oxidation of the cystine in the food. The excretion of neutral sulphur, however, is much less affected by the amount of protein ingested and therefore, very probably is derived from endogenous metabolism or from the break-down of body tissue, as also from sulphur existing in the bile. This theory is supported by the fact that when cystine is fed it is chiefly excreted as inorganic sulphates while ingested taurine appears mostly as neutral sulphur. The inorganic sulphates, consisting of sodium, ammonium and potassium sulphates, are merely the neutralization products of the sulphuric acid formed by complete

oxidation of the cystine or cysteine present in food. The ethereal or conjugated sulphates, finally, are organic sulphates of the nature of an ester, resulting from the union of an alcohol with sulphuric acid. As an example, we have from the work of Baumann and Herter (8) phenol-potassium-sulphate:



This was the first member of this class of substances to be studied, but since that time more than 200 such compounds have been found. The best known of them are indican (indoxyl-potassium-sulphate), cresol-potassium-sulphate and skatol (skatoxyl-potassium-sulphate), which are the detoxication products of compounds resulting from intestinal putrefaction. The ethereal sulphates in the urine amounting to 0.1 to 0.3 gram per day form roughly 10 per cent of the total sulphate output.

The simplest explanation of the formation of ethereal sulphates is no doubt this, that they arise by a direct combination of the organic base with the inorganic acid salt. Thus when phenol is fed in small quantities it merely joins on to potassium hydrogen sulphate before the second atom of potassium combines with the acid salt to form normal potassium sulphate. That ethereal sulphates are actually formed at the expense of the inorganic sulphates is evidenced by the fact that in cases of phenol and cresol poisoning, where the available supply of sulphuric acid is insufficient for the large amount of poison ingested, the entire output of oxidized sulphur appears in the form of ethereal sulphates to the total exclusion of the inorganic portion. Still, that is not the only explanation of their origin, as can be seen from the following facts. When brombenzol is fed much of it is eliminated in the urine in combination with an acetylated cysteine molecule. The brombenzol, it is believed,

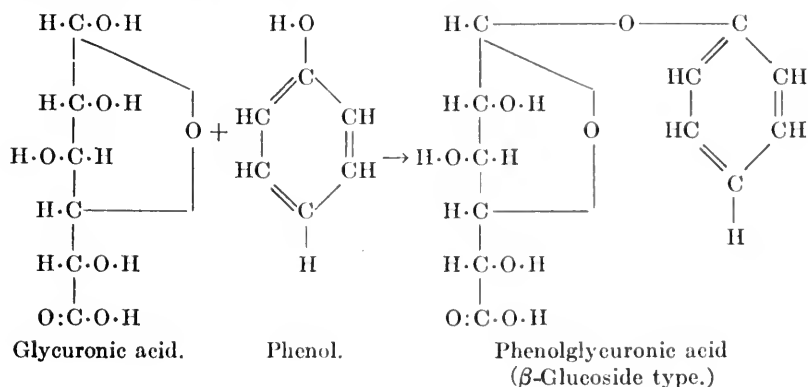
is first oxidized to p-bromphenol, which, being an alcohol should combine with sulphuric acid and add to the amount of ethereal sulphates excreted. As a matter of fact, however, combination is effected in this instance through the reduced sulphur group of cysteine. Very probably, therefore, the synthesis of bromphenylmercapturic acid represents a step in the intermediary metabolism of cysteine. Furthermore, it is very likely a type for many similar cases, with this one difference, that in the other instances the resulting compounds appear later on as ethereal sulphates, whereas in the case of p-brom-phenyl-cysteine, for some reason or other, oxidation to a sulphate is, at most, only partial. As a matter of fact, it has been found that after feeding brombenzol there is always an increase of 600 to 1000 per cent in the output of ethereal sulphates and a decided decrease in the amount of inorganic sulphates excreted. This would seem to indicate, therefore, that possibly some of the bromphenylmercapturic acid had been oxidized to p-bromphenylsulphuric acid. There is also an increase in neutral sulphur after feeding brombenzol, but on careful perusal of the data (35), (67), it is easily seen that this is due to the addition of the mercapturic acid sulphur to the normal quota of reduced sulphur and that the latter is not really affected at all.

One would conclude from these experiments either that the ethereal sulphates are directly produced at the expense of the inorganic sulphates by forming esters with sulphuric acid or acid sulphates already at hand, or that they arise from a cysteine conjugate. In the latter case the resulting compound, the analogue of bromphenylmercapturic acid, would undergo oxidation to form the sulphates. The cysteine for this purpose is no doubt mostly of exogenous origin, for mercapturic acid can not be synthesized on a non-protein diet. One might be led to think that ethereal sulphates are also dependent on exogenous cysteine for their formation. Folin, however, has shown that this is by no means the case for he found that the decrease in the ethereal sulphate output on a low protein diet was only relative. The question cannot be fully answered, however, without taking into consideration the sulphur metabolism of the bacteria residing in the intestinal tract.

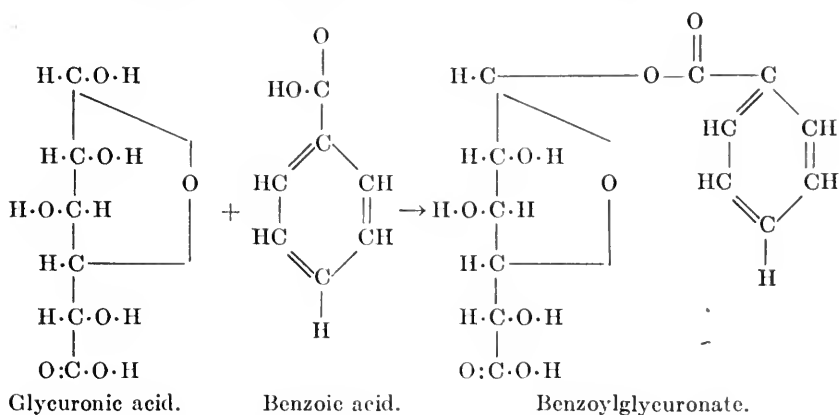
**GLYCURONIC ACID.** This substance is found in combination with an almost unlimited number of compounds, but when the matter is considered as a whole, it is seen that most of these substances contain a hydroxyl group and may be classed, therefore, as aliphatic or aromatic alcohols. Primary and secondary alcohols are quite easily conjugated with glycuronic acid, for which reason many aldehydes or ketones when

fed are excreted in this form of combination after undergoing a reduction to their corresponding alcohols. Although some of the primary and secondary alcohols do not combine with glycuronic acid, practically all the tertiary alcohols appear to do so in the organism of the rabbit.

There are at least two different forms of glycuronic acid combinations and present indications point to a probable third. The first is a *glucoside type* of binding, which often appears as pictured below in cases of phenol poisoning (79) (86).



The second or ester type of union is found in such combinations as that formed between benzoic and glycuronic acids (47) (64).



To the glucoside type belong the compounds of glycuronic acid with the alcohols and phenols, while the second type is employed in the case of certain toxic organic acids, notably benzoic acid, dimethylamino-

benzoic acid, salicylic acid and very likely the halogen phenylmercapturic acids. The ester type of glycuronates reduces Fehling's solution, but the glucoside type will effect no reduction until the compound has been hydrolyzed. The glycuronic acid, which is in itself a strong reducing agent by virtue of its aldehyde group, is thus set free.

There is still much difference of opinion regarding the source of glycuronic acid, as to whether it is formed from glucose and if so whether it is a normal decomposition product of that substance, or whether it originates entirely independently of glucose metabolism. It is rather unreasonable to suppose that this carbohydrate-like compound should be directly synthesized by the body when it could be obtained so simply from glucose. On the other hand, it seems highly improbable that it is a normal catabolic product of glucose since the aldehyde group, which would certainly be the first point of attack when the glucose molecule is subjected to oxidation, is still present in glycuronic acid. Furthermore, the fact that after ingestion of large amounts of glycuronic acid we find gluconic acid, saccharic acid and much oxalic acid in the urine means simply this, that glycuronic acid is oxidized with difficulty, but does not indicate that it is a normal intermediary product of glucose catabolism. Perhaps the most plausible explanation of the origin of glycuronic acid is this, that a union is effected between the aldehyde group of glucose and the foreign molecule, by which the former is protected against further oxidation. The next point of attack then is the primary alcohol group which is converted accordingly into a carboxyl.

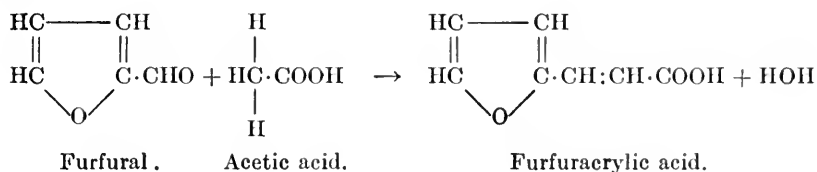
A mass relationship always exists between sulphuric acid and glycuronic acid when there is question of effecting combination with the aromatic substances which are formed in intestinal putrefaction. If the concentration of either of these acids is increased the amount of conjugation with the aromatic poisons will be varied in the direction of the higher concentration.

ACETYLATION. Acetic acid not infrequently, plays an interesting and perhaps important rôle in intermediary metabolism. The usual type of reaction into which it enters is the formation of an acetylamino compound.

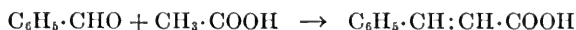


Under the treatment of reduction, a case of acetylation has been mentioned in which acetylamino benzoic acid was formed. Still another

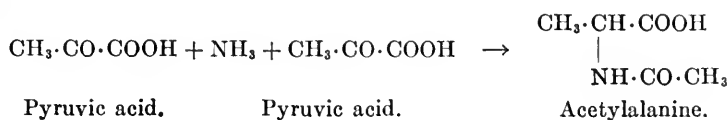
case was that of the formation of p-bromophenylmercapturic acid. Knoop and Kertess (55) have demonstrated the possibility of acetylation of an  $\gamma$ -amino acid when they recovered  $\gamma$ -phenyl- $\alpha$ -acetylaminobutyric acid after feeding dl- $\gamma$ -phenyl- $\alpha$ -amino-butyric acid. Likewise dl-phenylaminoacetic acid is excreted to some extent as d-phenylacetylaminacetic acid (78). Perhaps the most striking case of the entrance of acetic acid into one of these reactions was that reported by Jaffé and Cohn (49). After feeding furfural to dogs and rabbits, they found that the methyl group of acetic acid had condensed with the aldehyde group of furfural resulting in the formation of fufuraacrylic acid.



This is exactly analogous to the formation of cinnamic acid by the Perkins reaction from benzaldehyde, acetic anhydride and sodium acetate:



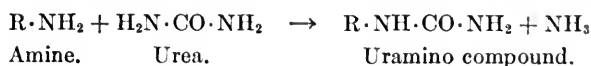
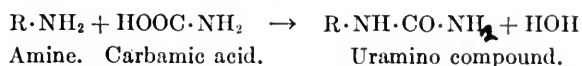
It was formerly believed that acetylation was extremely complicated and was brought about only in connection with certain simultaneous processes of oxidation. This view, however, has since been disproved by Ellinger and Hensel (23) who showed that both p-aminobenzaldehyde and p-aminobenzoic acid were acetylated in the organism of the rabbit as completely as p-nitrobenzaldehyde. The reaction occurred, therefore, independently of either oxidation or reduction in the molecule. The more recent work of Miss Hensel (38) on the quantitative studies of acetylation in the animal body tends to confirm the earlier views of Knoop (53) (55) who believed that acetylation is probably produced from pyruvic acid and ammonia. If, for example, two molecules of pyruvic acid interact with one molecule of ammonium carbonate with the formation of carbon dioxide and water, we have formed acetylalanine:





little can be learned regarding the nature of the reaction. The formation of methylamino compounds in the plant world is very common, but the manner in which they are produced is still a mystery. It has been suggested that the plant accomplishes the reaction by means of formaldehyde and amino groups, but as we have absolutely no proof of the presence of formaldehyde in animal tissues, this explanation is hardly tenable in the case of animal methylation.

**URAMINO ACIDS.** Amino compounds have been isolated in which the amino group either in the aromatic nucleus or on the side chain has formed an additive compound with a  $-\text{CONH}_2$  rest. This may be considered as the reaction product of the amino group with carbamic acid,  $\text{NH}_2 \cdot \text{COOH}$ , or with urea,  $\text{NH}_2 \cdot \text{CO} \cdot \text{NH}_2$ , forming respectively water and ammonia as by-products.



In the laboratory this synthesis is effected by treating the amino acid with HCl and potassium isocyanate. It is very probable, therefore, that most of the uramino compounds found in urine are not produced within the organism but are formed during the evaporation of the urine by the interaction of the amino compound with urea. Some of the best known examples of uramino acids are m-uraminobenzoic acid and taurocarbamic acid. The latter, a union of  $-\text{CONH}_2$  and the amino group of taurine, was thought at one time to be a compound of considerable importance, until Schmidt and Allen (90) showed that taurine actually passes through the body unchanged but on evaporation of the urine combines with urea to form the uramino acid. From time to time uramino acids have been reported, particularly side chain compounds, which crystallized from the unevaporated urine on standing. These very probably were products of synthetic reactions which occurred within the body. In general, one may say that the uramino acids are very likely of much less importance than at first considered.

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## THE PHYSIOLOGICAL ACTION OF LIGHT

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The first systematic effort to study the biological effects of light, and its therapeutic uses, was made by Finsen (1) when he founded his Light Institute in Copenhagen in 1896. Much valuable work, both theoretical and practical, has been done there since, with especial success on the therapeutic side, in the treatment of lupus, but the fundamental problem of the mode of action of light on the living cell remains unsolved. Recently, the rapidly accumulating clinical results of light treatment in tuberculosis, rickets, malaria, etc., closely related as they are to the results of x-ray and radium treatment, continually emphasize the importance of this problem and increase its mystery.

It is at first disappointing to find that there is, apparently, in the animal kingdom no effect analogous to the action of light on the chlorophyll system of the green plant, by means of which light energy is stored and oxygen restored to the atmosphere. Although there is a universal conviction that sunlight is healthy, it is certain that people and animals can live a long time in darkness without any noticeably bad results. Blessing (2), who acted as physician to Nansen during his expedition in the Fram, published a report showing that members of the party exhibited no evidence of anemia during the trip. More recently, Grober and Sempell (3) examined horses that had worked for years in coal mines and found no anemia in any case where a satisfactory nutritive condition existed. But, though the physiological effect of sunlight seems at first sight indefinite and of dubious importance, the action of far ultraviolet light on normal tissue, and the action of near ultraviolet and visible light under certain pathological conditions, has been investigated enough to show that there are well-defined effects due to light, closely related to the physiological results of exposure to radium and x-rays. These results are gradually assuming considerable importance in clinical medicine and present theoretically an interesting but illusive problem in physiology. This review will give as briefly as possible the facts that have been experimentally proved so far with a discussion of the theoretical and practical considerations suggested by them.

*Effect of light on microorganisms.* The most definite result of the early work on the action of light on the living cell was the proof that ultraviolet light exerts a strong lethal action on bacteria. In 1877 Downes and Blunt (4) showed, for the first time, that sunlight retards the growth of bacteria and proved that this was not due to heat since the same result was obtained with tubes cooled in ice.

Marshall Ward (5) in 1893 exposed anthrax bacilli, in gelatine media, to sunlight and, by means of color filters, demonstrated that the effect was due to the violet end of the spectrum. With a solar spectrum projected on the inoculated media the effect was found to begin in the greenish blue.

In 1905 Hertel (6) did the first quantitative piece of work in this subject. He examined the physiological effect of rays of different

TABLE I  
*Time for lethal action of light*

WAVE LENGTHS $\mu\mu$	GALVANOMETER DEFLECTIONS			
	10	50	500	
232	60''	10''		Bacteria
280	3'	40''	7''	
334		60''		
383		8'		
440			3 hrs.	
280	3'			Paramoecia
334		14'		
440			3 hrs.	

wavelengths and the same energy, the energy of the light being measured by means of a thermocouple and galvanometer. His results show conclusively that the shorter the wavelength the greater the lethal effect on bacteria and paramoecia (table I).

As the spectrum of sunlight reaches only to  $290\mu\mu$  in the ultraviolet, greater germicidal effects can be obtained by means of artificial sources, such as the quartz mercury arc and bare metallic arcs. In a recent paper by Browning and Russ (7) a tungsten arc was the source of light used. This gives a rich line spectrum extending as far as  $210\mu\mu$ . A gelatine plate was inoculated with microorganisms instead of being sensitized with a silver salt, exposed to the spectrum of the arc through a quartz spectrograph, and incubated at  $37^\circ$ , with the result that bacteria exposed to wavelengths of  $296\mu\mu$  or less were killed. This paper

would seem to show that sunlight contains few rays short enough to affect bacteria. But wavelengths somewhat longer than  $296\mu\mu$  are lethal with longer exposure or with exposures at a higher temperature. Thiele and Wolf (8) found that wavelengths greater than  $300\mu\mu$  were harmless to bacteria at a temperature of  $14^{\circ}$  to  $20^{\circ}\text{C}$ . and lethal at  $30^{\circ}$  to  $40^{\circ}\text{C}$ . In Ward's experiments the temperature was probably such that the lethal action began in the greenish blue (about  $480\mu\mu$ ). No exact experiments have been made as yet giving the upper wavelength limit of the effect at different temperatures.

There is, however, in all the physiological actions of light, a very marked difference between the effect of light from 400 to  $300\mu\mu$  (near ultraviolet) and light from 300 to  $100\mu\mu$  (far ultraviolet). Light greater than  $300\mu\mu$ , being our normal environment, it is obvious that any organism, ordinarily exposed to this light and easily injured by it, would have perished long since. Light less than  $300\mu\mu$  is an unnatural environment and produces in all living cells strong and often very harmful reactions. Since the effect of light is probably due to the photochemical reactions produced when light energy is absorbed it is not surprising to find that the various constituents of protoplasm begin to absorb light strongly in the neighborhood of  $300\mu\mu$ . Much careful and valuable work on the absorption of substances in the ultraviolet has been done by Henri (9). He finds that the abiotic power of light is almost exactly proportional to its extinction coefficient. When the organisms are small the entire protoplasm is affected and the action obeys the laws of simple photochemical reactions. If the organisms are large the effect is a surface one, owing to the small penetration of the ultraviolet light. In the latter case the protoplasm affected may, by a process of diffusion, affect the rest and the process is similar to a complex photochemical reaction taking place at a strongly absorbing surface.

We do not know the exact nature of the photochemical reactions produced in protoplasm by ultraviolet light, although various clues have been suggested. Bovie (10) finds that paramoecia exposed to a sublethal dose of ultraviolet light are so sensitized to heat that they can not stand, even for sixty seconds, a temperature which is an optimum for the controls. He concludes that death from ultraviolet is due to heat coagulation following sensitization by radiation.

Schanz (11) has shown that the effect of ultraviolet light on protein solutions is to make them less soluble, as indicated by their easier precipitation. Chaluppecky (12) finds that exposure of egg and serum

albumin to ultraviolet light results in an increase in the globulins at the expense of the albumins. Recent experiments in this laboratory show that a dilute solution of egg albumin may be changed so as to react like globulin towards ammonium sulphate after an hour's exposure, in a quartz test tube, to the light of a quartz mercury arc at a distance of 5 cm. Through glass the light has no effect.

There is also some evidence that lipoids are rendered more soluble on exposure to light, thereby possibly explaining the hemolytic action of ultraviolet light on a suspension of washed red corpuscles (13).

Bovie (14) has also shown that a sublethal dose of ultraviolet light inhibits the cell division of paramoecia. He used a magnesium spark through quartz, which gives a strong line at  $280\mu$  and obtained the following interesting results. 1, There is inhibition of cell division with  $\frac{1}{2}$  the exposure necessary for cytotoxicity. 2, The duration of the inhibition increases with increasing exposure. 3, The inhibition is followed by an acceleration of cell division which may, for a short exposure, give an increase in the number of radiated individuals over the controls. Shorter wavelengths from a hydrogen tube, filtered through fluorite, gave a strong lethal action but no inhibition of cell division. The reason given for this is that these very short rays are absorbed in 3 to 4 microns of protoplasm and do not reach the nucleus.

Another result of sublethal exposures on microorganisms is reported by M. and Mme. Henri (15). They found it possible, by means of short exposures to ultraviolet light, to transmute anthrax bacilli to cocci, with various intermediate forms. The modified forms, unlike the original bacilli, can obtain their nitrogen from ammonium salts, or the amino acids, and develop best in media containing sugar. They conclude from this that exposure to ultraviolet light destroys the power of the bacilli to secrete proteolytic enzymes while leaving uninjured the ability to form amylolytic enzymes. As, however, the modified form returns to normal on inoculation into animals, there has been no fundamental change produced and the results are of dubious importance. It is, however, interesting to note that they are in direct contradiction to Burge's work (16) in which he killed bacteria by exposure to ultraviolet light, ground them up to extract the enzymes and found the proteolytic enzymes unharmed. The question of the action of light on bacterial enzymes is evidently in need of further investigation.

By means of its lethal action on bacteria, and also on protozoa (17), the sun is of undoubted hygienic value to mankind. But it is probably beneficial in a less indirect way as well, as there is growing evidence to show that light may have a direct action on higher animals.

*Effect of light on the eye.* Since the eye is the specialized organ of light response it is possible, as suggested by Browne (18), that there may be changes in metabolism due to a reflex stimulation through the eye. In support of this view he cites the chromatic adaptation of certain frogs and fishes through the eye. There is also some experimental evidence that rabbits give off more  $\text{CO}_2$  (19) in the light than in the dark, and that when blinded the output of  $\text{CO}_2$  is the same. However, this theory is unlikely since most striking physiological effects are produced in higher as well as in lower animals by far ultraviolet light from which the eye must be carefully shielded. The direct effect of light on the eye can be given briefly as follows. Light of wavelength  $760\text{--}380\mu\mu$  (the lower limit being variable and depending to some extent on the age of the individual) penetrates to the retina and is perceived as visible light. Light from  $380\text{--}295\mu\mu$  is absorbed by the lens and causes it to fluoresce. Light of wavelength shorter than  $295\mu\mu$  is absorbed by the cornea and conjunctiva producing a severe ophthalmia.

The absorption of the eye media for infrared light or heat radiation (40) is equivalent to the absorption of 2.28 cm. of water. Although it is possible that heat may be a contributing cause in glass blower's cataract, there is no evidence of injury to the eye by heat except in eclipse blindness, which is probably due to heat coagulation of the retina (21). Also, visible light probably produces no lasting ill effect on the retina, except in so far as it contributes to the heat effect in eclipse blindness. The discomfort and disturbance of vision caused by the glare of bright light sources is a most complex question which is not well understood at present. It is largely a question of the amount of contrast in the field of view, for with properly diffused light the eye can function comfortably in bright sunlight, which gives an illumination of several thousand foot candles.

Light from  $295\text{--}385\mu\mu$ , although absorbed by the lens, produces no ill effect as a rule. Most proteins are coagulated by the ultraviolet light which they absorb but the lens protein seems to be resistant in this respect, at any rate for the wavelengths  $295\text{--}380\mu\mu$ . The light absorbed by the lens causes a strong fluorescence in it and Burge (22) suggests that the fluorescence constitutes a protective reaction, but the nature of the protection is not clear. It may be mentioned, as a matter of interest, that in some nocturnal animals (rats, mice) the lens does not fluoresce to waves of this length. Burge (22) has shown by a series of ingenious experiments that, in the presence of certain salts, lens proteins coagulate with light longer than  $300\mu\mu$  and he supposes that a combi-

nation of nutritive disturbances and strong light may account for the development of cataract. With this possible exception, however, the light absorbed by the lens is harmless.

Far ultraviolet rays are all absorbed by the conjunctiva and cornea. Light of this wavelength (less than  $295\mu\mu$ ) results in a severe conjunctivitis and, with a long exposure, corneal ulcers are formed. Artificial lights, if glass covered, are therefore harmless and sunlight rarely contains enough far ultraviolet to produce injury. However the ultraviolet reflected from large areas of water and from snowfields may result in the temporary injury known as snowblindness. Artificial illuminants, which emit a large amount of radiation of wavelength less than

TABLE 2  
*Transmission of light by the skin*

	WAVE LENGTH ( $\mu\mu$ )							
	436	405	366	354	313	302	297	289
Per cent transmitted by skin 0.1 mm. thick.....	59	55	49	42	30	8	2	0.01
Per cent transmitted by skin 1.0 mm. thick.....	0.5	0.3	0.08	0.02				

TABLE 3  
*Thickness of protoplasm absorbing 90 per cent of light*

Thickness of protoplasm ( $\mu$ ).....	79	58	18	9	6	3.8
Wavelength ( $\mu\mu$ ).....	240	238	231	226	219	214

$295\mu\mu$ , such as the quartz mercury arc and bare metallic arcs, are known to be extremely injurious and the eyes should be carefully protected from them.

*Effect on the skin.* It is difficult to give exact figures as to the extent to which light of different wavelengths penetrates the skin, although a number of papers have been published on the subject. The following quantitative results have been given by Hasselbalch (23) (table 2).

Henri (9) gives the thickness of protoplasm which absorbs 90 per cent of ultraviolet light of different wavelengths (table 3).

Giltcher (24) gives a curve combining his results with those of Hasselbalch, which is shown in figure 1. Roughly one can say that light less than  $300\mu\mu$  is absorbed by the epidermis in a layer 0.1 mm.

thick. The shorter the wavelengths the smaller the layer that will completely absorb them. Blood serum absorbs everything below  $300\mu$  (25). Normal blood absorbs all wavelengths less than  $450\mu$  and has two absorption bands at  $540$  and  $575\mu$ . Bloodless tissue is found to transmit some light throughout the visible spectrum.

As a result of the absorption of ultraviolet light in the epidermis, the familiar inflammatory reaction known as sunburn is produced. The end result of this inflammation is the deposition of the pigment melanin

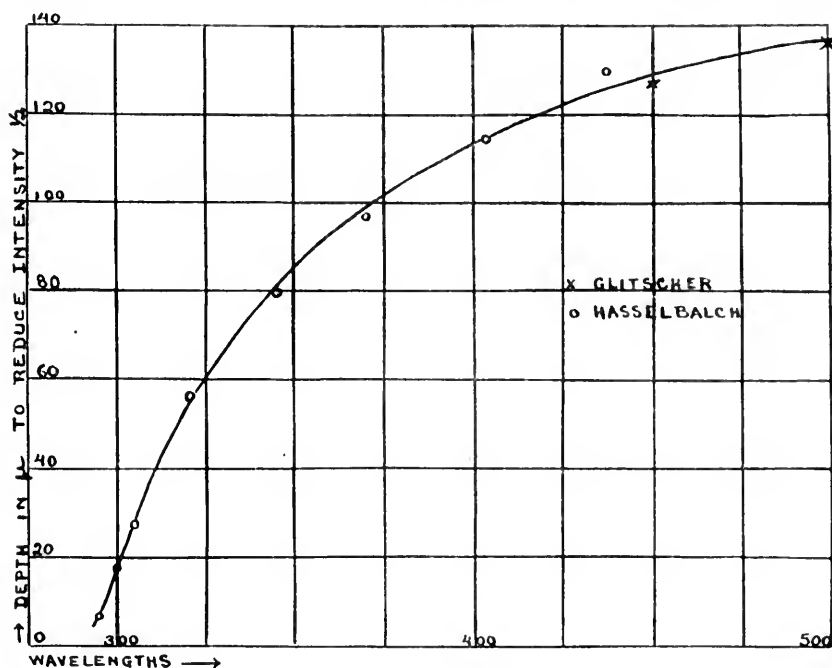


Fig. 1. Absorption of light by the skin.

in the basal cells of the epidermis. Melanin is assumed to be the end product of the oxidation of tyrosin or of one of the related cyclic compounds, probably through the agency of an oxidizing ferment such as tyrosinase. It is not known how light initiates this reaction and there are many theories as to the rôle that pigmentation plays in human physiology. There is a widespread conviction, not however based on very accurate observations, that people who do not tan respond badly to heliotherapy. Rollier (26), whose alpine sanitarium for the light treatment of tuberculosis has had such great success, is convinced that

the beneficial action of sunlight is due to the pigmentation produced. He has a theory that pigment, by fluorescent action, transforms short lethal rays to long non-lethal rays of a greater penetration, thereby doing away with any danger from far ultraviolet light and increasing the depth of light absorption. There is no experimental basis for this theory, and on examining the human skin under Wood's invisible monochromatic light (wavelength  $366\mu\mu$ ), which produces the most beautiful fluorescence in all substances possessing that property, pigmented areas, such as freckles, are found to be entirely non-fluorescent. It is possible that the chemical change taking place in the epidermal cells, resulting in the formation of pigment, acts in some way as a stimulus to metabolism. The question cannot be definitely discussed since little is known about these chemical changes and one can not definitely say just which rays are responsible for the formation of pigment. It is formed, we know, by the ultraviolet radiation in sunlight so that wavelengths greater than  $290\mu\mu$  are effective and experiments by de Laroquette (27) show that sunlight through glass, which cuts off wavelengths less than  $330\mu\mu$ , produces only a slight degree of pigmentation. The most effective rays in sunlight seem, therefore, to be between  $290$  and  $330\mu\mu$ . Jungling (28), although he shows that solar erythema is only produced by rays less than  $330\mu\mu$ , the effect increasing rapidly with decreasing wavelength, finds that pigment can be produced by rays greater than  $330\mu\mu$  and that rays less than  $290\mu\mu$ , while producing a violent inflammation, do not result in heavy pigmentation. Apparently these very short wavelengths are absorbed before they reach the basal cells of the epidermis and are therefore less effective than the ones from  $290$  to  $330\mu\mu$ . Owing to the difficulty of filtering ultraviolet light so as to obtain only rays less than  $290\mu\mu$ , it is not known how short a wavelength is able to produce pigmentation.

It is undoubtedly true that one function of pigment is to act as a protection to the underlying tissues against too much radiation. But at the same time it insures a maximum absorption of radiant energy in the basal cells of the epidermis and may therefore result in an increased light reaction at that point. Ultraviolet light of wavelength shorter than  $290\mu\mu$  would not reach these basal cells except where the epidermis is less than  $0.1$  mm. thick, so that pigment affords no protection against radiation shorter than that found in sunlight. Any speculation on the rôle played by pigmentation suggests a comparison between the response of white people and negroes to heliotherapy. As nearly all of the published work on heliotherapy is foreign, and most of it German, this comparison has not yet been made, so far as the writer knows.

*Light as a stimulus to plain muscle.* Adler (29) has experimented with the effect of light on surviving organs and finds that ultraviolet radiation can act as a stimulus to the stomach and intestines of the frog, and to the uterus of the rabbit and guinea pig. Visible light has no effect unless the organs are sensitized with some dye such as eosin or hematoporphyrin (see section on photodynamic sensitization), in which case it acts as a stimulus also.

*The effect of light on the blood.* Numerous investigators have studied the effect of light on the blood and their results, although usually complicated by the effects of heat and altitude, agree fairly well. The effect of sunlight on the erythrocyte count is not very marked. For short exposures there is no response and some observers have reported negative results when the exposures extended over a considerable length of time (30). However, enough observers have obtained positive results to make one believe that there is a decrease in the red count and the percentage of hemoglobin in the dark and an increase in the light, after long exposures (31), (32), (33). These changes are not permanent and the blood count later returns to normal (2), (3).

On the contrary, the white blood cells, especially the lymphocytes, respond to short exposures of any radiation (sunlight, ultraviolet light, x-ray, heat). Murphy (34) has shown that a 5-minute exposure to dry heat at 55° to 65°C. will produce a rise in lymphocytes of 100 to 200 per cent over the normal count. Russ (35), Taylor (36) and others have shown that on exposure to x-rays the lymphocytes fall rapidly. The response to light is less striking but certain definite results have been found and all the published results agree in the conclusion that ultraviolet light stimulates a lymphocytosis in men and animals (37), (38), (39), (40). In animals it is found (40) that this ultraviolet lymphocytosis is due entirely to rays shorter than 330 $\mu$ m. Ultraviolet light from 330 to 390 $\mu$ m diminishes the lymphocyte count slightly but light from a metallic arc very rich in wavelengths shorter than 330 $\mu$ m produces a marked lymphocytosis reaching its maximum in about five days. The production of lymphocytosis, as well as the formation of pigment by ultraviolet light may well be an important factor in heliotherapy. The means by which this lymphocytosis is produced is as yet a mystery. Bunting and Huston (41) have recently published experiments showing that more lymphocytes enter the blood stream from the thoracic duct during twenty-four hours than are present in the blood at any time. The excess of cells migrates from the blood vessels into the mucous membranes, chiefly the gastro-intestinal tract. A

lymphocytosis might result either from an increased rate of production or from a decreased rate of destruction, probably from the former. Presumably the lymphocyte-forming organs are stimulated to greater activity by some photochemical change produced by ultraviolet light. Blood radiated outside the body, and then introduced into the blood stream, has no effect on the lymphocyte count (40), so that whatever the photochemical reaction may be it probably takes place in the surface tissues through which the blood circulates, or in the walls of the capillaries themselves.

Most interesting effects of ultraviolet light on the internal organs have been reported by Levy (42) and Gassul (43). Mice were radiated with ultraviolet light for periods ranging from 10 minutes to 56 hours. The animals were then killed and the internal organs examined both macroscopically and microscopically. The spleen, lungs, and liver were found engorged with blood. The spleen was most affected, having increased to two or three times its normal size, partly due to a very great hyperemia, partly to the development of large masses of connective tissue around the follicles. It is apparent then that the absorption of ultraviolet light in the surface of the body to a depth of about 0.1 mm. results not only in an immediate or direct effect on the skin, as indicated by its inflammation and pigmentation, but also in an indirect or distant effect on such organs as the spleen and lungs, which can be explained only by a cutaneous reflex of some sort, or, more probably, by the assumption that some photochemical product, formed at the surface, is conveyed to the internal organs by the blood. The congestion of the lungs was such that the red cells had passed through the capillary walls into the alveoli. This may explain why heliotherapy in lung tuberculosis is frequently followed by hemorrhage.

Ultraviolet light has a hemolytic action on a suspension of washed red corpuscles, outside the body. In the presence of serum this effect does not take place. The protective action of serum, according to Schmidt and Norman (44), is due to certain amino acids, especially tyrosin and tryptophan, owing to the fact that these substances absorb the far ultraviolet. There is no evidence of this action *in vivo*, probably owing to the protection afforded by the skin and serum.

*Effect on metabolism.* Light exerts some influence on body metabolism, as is shown by a number of results indicating a change in amount of  $\text{CO}_2$  expired, a change in rate and depth of respiration, and an increased rate of growth in the light compared to the dark. However, no results definite enough to review at any length are reported in this field. Since

ultraviolet light is a powerful oxidizing and reducing agent it seems strange that it does not produce more marked changes in metabolism than it does. The small penetration of the chemically active rays is of course our protection against them. As far as the change in  $\text{CO}_2$  output is concerned, it is visible light and ultraviolet light greater than  $330\mu\mu$  which is responsible for the effects reported so far, as the animals experimented on were always exposed to light under glass. None of these experiments are recent (19), (45), (46), but they suggest that it would be worth while to study the basal metabolism of human beings in the light and the dark, and in light of different wavelengths. Very recently experimental work on rickets (66), (67) has given evidence that light is concerned in the phosphorus and calcium metabolism of the body. The results of this work are discussed further under the section on heliotherapy.

*Photodynamic sensitization.* Although ultraviolet rays, particularly those below  $300\mu\mu$ , produce many reactions in living cells, visible light is apparently without any effect except in producing vision, heat, and possibly some change in metabolism. It is however possible to sensitize living cells, just as one sensitizes a photographic plate, and produce an abnormal condition in which visible light is as active as ultraviolet. This phenomenon has been called photodynamic sensitization. It was discovered accidentally by Raab (47). Under the direction of von Tappeiner he was studying the toxic action of acridin on paramoecia and the discordant results in determining the minimum fatal dose led to the discovery that acridin is lethal only in the light. With a strength of 1:20000, paramoecia are killed in 6 minutes in direct sunlight, 1 hour in diffuse daylight, and are unharmed in the dark. After this fact was discovered the subject was extensively investigated by von Tappeiner, Jodlbauer, and their co-workers (48) and many substances were found to act as sensitizers, fluorescein and its derivatives being especially potent. In vitro, a surprising number of interesting results was obtained and it was found possible to sensitize, to the action of visible light, bacteria, protozoa, red blood corpuscles, enzymes, ferments, the various substances concerned in immunity, and certain well-defined chemical substances, such as the combination of mercuric chloride and ammonium oxalate.

Von Tappeiner laid great stress on the fact that most of the substances which act as sensitizers are fluorescent. Photodynamic action however is not proportional to the degree of fluorescence and, although only that region of the spectrum is effective which is absorbed by the fluorescent

substances (bluish green for eosin, violet for quinine), it is not always the region that gives the strongest fluorescence that gives the greatest photodynamic sensitization. Also a number of non-fluorescent substances act as sensitizers of photographic plates. Von Tappeiner considers this a different phenomenon, but as Sellards (49) says, in his interesting review of the subject, it is hard to see why one should make any distinction between the oxidation of substances such as KI or metallic silver by eosin and light, and the acceleration of a photographic plate by chlorophyll.

The light emitted by the fluorescent body is in itself ineffective. It is necessary that the sensitizer be in contact with the material on which it acts. Von Tappeiner probably laid a great deal too much stress on the importance of fluorescence in photodynamic action. It is the usual accompaniment but not the fundamental cause of the sensitization, a sort of outward and visible sign, so to speak, of an inner activity in response to light. Fluorescence, in fact, is a more general property than is usually supposed. In a dark room, under Wood's invisible light (quartz mercury arc through a filter transmitting only wavelength  $366\mu\mu$ ), nearly all substances show some degree of fluorescence.

There is no evidence that the sensitizer is permanently altered by exposure to the light. If eosin is exposed to light and then added, in the dark, to a solution containing bacteria, or washed red blood corpuscles, it is as harmless as if it had never been exposed. The presence of oxygen is necessary for most of the effects produced by visible light and the only light which is effective is light of wavelengths corresponding to the absorption band of the sensitizer.

The sensitizing reactions are specific. Although eosin seems to be a sensitizer for all cells and chemical substances, this is not invariably true of other dyes. For instance, methylene blue acts as a sensitizer to katalase but not to peroxidase, and the relative sensitizing action of various dyes is different towards different substances. The specificity of the phenomenon is especially striking when the experiments are tried in vivo. Although a great many substances sensitize in vitro, only eosin, chlorophyll and certain derivatives of hemoglobin have so far been found effective in vivo and the only markedly effective sensitizer for higher animals is hematoporphyrin. This substance is derived from hematin by removing the iron. It has the formula  $C_{34}H_{38}O_6N_4$ , but is usually obtained and used in its crystalline compound with hydrochloric acid. In vitro it is a less effective sensitizer than the members of the fluorescein group, but in vivo it produces the most marked effects.

Hausmann (50) injected white mice with it and found 0.01 gram harmless in the dark while 0.002 gram will bring on acute symptoms in the light. There is a marked but temporary hyperemia of the ears, nose and tail, and after a period of great activity the animal becomes quiet, shows dyspnea and dies in one to three hours. If exposed to a less intense light or if exposure to an intense light takes place some time after injection, the mouse develops a subacute form and dies in one to two days. With still less light, or a longer interval between injection and exposure, the animals develop a chronic form and remain sick and light-sensitive for months. The blood of the hematoporphyrin mouse is not hemolysed and it is probable, from the length of time that an animal remains sensitized, that hematoporphyrin forms a stable photosensitive compound with some element of the skin tissue which is only slowly broken down and eliminated.

As mentioned above, Adler (29) found that visible light acts as a stimulus to plain muscle in various organs, when they are sensitized with different dyes, and Amsler and Pick (50) have made numerous experiments with light on the surviving frog's heart. Neither eosin, nor hematoporphyrin, nor visible light alone affects the surviving heart injuriously but if a small amount of sensitizer is added to the perfusing solution, and the heart then radiated with visible light, the illumination produces a disturbance in the atrio-ventricular transmission of the impulse, resulting in a sort of heart block, in which the nervous rather than the muscular parts of the organ are affected.

There are a few instances of spontaneous sensitization in which the skin is abnormally sensitive to light. Buckwheat poisoning, among animals, is the best instance and it has been suggested that the skin lesions in pellagra indicate a light-sensitive condition in man. Meyer-Betz (52), with more devotion to science than to his own welfare, tried the effect of hematoporphyrin on himself, by injecting 200 mgm. into his own blood. Subsequent exposure to light produced most distressing symptoms similar to those seen in the mouse and he remained light-sensitive for a long time. In the light-sensitive condition, known as *hydroa vacciniformis*, hematoporphyrin is found in the urine and it has been suggested that it is an example of naturally occurring hematoporphyrin sensitization, since the symptoms bear some resemblance to Meyer-Betz' reactions. However, Sellards (49) after working at the problem from many different angles came to the conclusion that, on the whole, sensitization plays but little part in the etiology or therapy of the diseases of man. Bile pigments are photodynamic *in vitro*, but

apparently the body can be overwhelmed with highly hemolytic pigments without suffering serious injury. There is, perhaps, some mechanism protecting the body against the hemolytic action of bile pigments which is not so efficient toward hematoporphyrin.

As has been explained above, the usual meaning attached to photodynamic sensitization is the production by visible light, plus a sensitizer, of the same reaction that ordinarily takes place under ultraviolet light. It has however been shown by Howell (paper not yet published) that there is another type of sensitization, in which dyes plus visible light produce an effect opposite to that produced by ultraviolet light alone. Fibrinogen, freshly prepared, coagulates spontaneously under ultraviolet light. If exposed through glass there is no spontaneous precipitation, but it coagulates on heating to 60°C. or on the addition of thrombin. On adding eosin, or hematoporphyrin, and exposing to either ultraviolet or visible light, the fibrinogen is not only desensitized to heat, so that it fails to coagulate on heating to 90°C., but also fails to react with thrombin. Whether the action of hematoporphyrin on higher animals belongs to this type of sensitization, or to that usually observed, is not at present known.

*Heliotherapy.* The results of heliotherapy are entirely empirical and not at all based on the experimental facts of light action as outlined above. They suggest, however, many valuable lines along which experimental work should be done in the future.

1. *Skin diseases.* Much use has been made of ultraviolet light in various diseases of the skin with favorable results in many conditions, but the most striking positive result is the work of Finsen (1) in the treatment of lupus vulgaris. By means of concentrated sunlight or arc light, from which the heat was eliminated as much as possible, he was able to cure lupus in the great majority of cases. The light destroys the diseased tissue and promotes the growth of the healthy tissue. The treatment is a long one but on the whole safer and more successful than treatment by x-rays. Eczema and acne are sometimes cured by light, and birthmarks may be improved, but the same or better results can be obtained with x-rays. One advantage of ultraviolet over x-ray treatment is that it is much safer. Even when the skin is severely burned no bad after-results are noticed.

2. *Tuberculosis.* In addition to its effect on tubercular skin infections, light has a markedly beneficial action on most of the other forms of tuberculosis, particularly the surgical types. In 1903 Rollier (26) founded a sanatorium at Leysin for the treatment of tuberculosis by

heliotherapy and his results, especially with surgical tuberculosis, are so promising that the method is gradually being adopted elsewhere. Rollier's treatment consists of sunbaths, beginning with a short exposure of the feet, and gradually increasing the area exposed and the length of exposure until the whole body, except the head, is exposed for several hours a day. This light treatment, combined with outdoor life and a high altitude results in a remarkably large percentage of cures. The following statistics, quoted from Gassul's monograph (53) cover 1129 cases, 652 of which were adults (table 4).

The most striking result is in bone and joint tuberculosis. Exposure to sunlight increases the rate of disintegration of cells damaged beyond repair, while stimulating the activity of normal cells, and acting also as a stimulus to recalcification. X-ray photographs, after the light

TABLE 4  
*Results of light treatment in surgical tuberculosis (Rollier)*

	CURED	IMPROVED
	<i>per cent</i>	<i>per cent</i>
Skin tuberculosis.....	81.25	18.75
Bone and joint.....	75.98	7.80
Glandular.....	89.80	5.20
Peritonitis.....	80.30	8.20
Genito-urinary.....	77.80	22.20
Kidney.....	52.94	33.00

Total mortality 0.9 per cent.

treatment, give striking evidence of the effect upon bone formation. According to Rollier, phalanges that have entirely disappeared may be so completely recalcified as to be indistinguishable, in radiographs, from normal tissue, and adults seem to be as easily affected as children. This means that, for early cases at least, the disease can be checked, and motion preserved in the affected joint, the gradual establishment of motion going hand in hand with the healing process. There is also a marked reaction in sinuses and ulcers. Under light treatment there is a profuse discharge followed by sloughing, formation of healthy granulations and healing. In tubercular lymph glands there is a gradual reduction in size and in broken-down glands the contents are frequently absorbed. There is absorption of effusions in the joints, and in the peritoneal and pleural cavities, which is especially noticeable in peritonitis. A recent paper by Hyde and Grasso (54) gives details of

Rollier's method and the results of its application at Perrysburg, N. Y. Their results, though slightly less favorable throughout, are roughly similar to those of Rollier, given in table 4, and indicate that heliotherapy can be successfully used for surgical tuberculosis even at low altitudes. They report the use of the quartz mercury arc on dark days but say that it is inferior to sunlight. There are no conclusive results on the exclusive use of the mercury arc so that it is impossible to say how far it may be used to replace sunlight. There is some evidence that mercury arc treatment is useful in intestinal tuberculosis but in lung tuberculosis both sunlight and artificial light baths are apt to lead to hemorrhages, possibly on account of the congestion of the lungs, which is known to follow exposure to ultraviolet light in the lower animals (42), (43).

How sunlight acts upon tuberculosis is only conjecture at best. Rollier insists on the fact that the benefit is always proportional to the degree of pigmentation. Without knowing accurately which wavelengths are responsible for pigmentation and which are most beneficial in the treatment of tuberculosis, any connection between the two effects is largely guesswork. It may be that the chemical changes resulting in pigmentation give a stimulus to the entire body metabolism. It may be that after it is formed the pigment acts as a sensitizer to light. On the other hand, the production of a lymphocytosis may be the important result. It is difficult to get a clear picture of the blood changes due to heliotherapy, as the blood picture in different types of tuberculosis is different to begin with, and the effect of altitude usually complicates the results. Rollier (26) states that, with heavy pigmentation and good prognosis, lymphocytosis takes place. Murphy (55) has found that mice with a high lymphocyte count are resistant to the inoculation of tubercle bacilli, so that the blood changes may well be the important ones. If this is correct, wavelengths less than  $330\mu$  should be the most effective in producing good results.

Gassul (53) in a recent monograph has given a summary of the results of treatment of tuberculosis with radiations of every type (light, x-ray, radium). Figure 2 is taken from his paper and shows most graphically the high percentage of cures possible by radiation treatment.

3. *Wounds.* In connection with the effect of light on tuberculosis it is interesting to note that a number of papers have been published on the use of ultraviolet light in the treatment of wounds (see review by Breiger (56)). The benefit may be partly due to the bactericidal action of the short wavelengths but there is also a marked effect similar to the

action on tubercular lesions. The diseased tissue is destroyed and the growth of healthy granulations stimulated.

4. *Rickets*. In tuberculosis of the bones and joints light is found to act as a recalcifying agent and this property makes it also a beneficial agent in rickets. A number of papers have appeared in foreign journals on this subject (57), (58), claiming that under light treatment, with the quartz mercury arc, the recalcification of bones proceeds at an accelerated rate, and that in spasmophilic rickets symptoms of tetany disap-

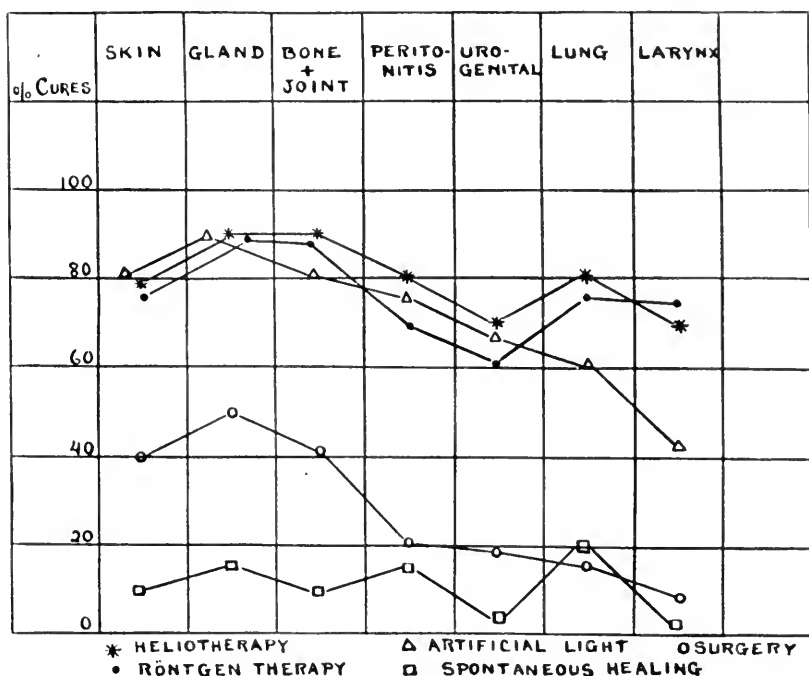


Fig. 2. Percentage of cures in surgical tuberculosis under different methods of treatment.

pear. These experiments were made on human subjects and all the evidence has been furnished by radiographs. The study of this effect has recently been taken up in this country in a more systematic way (59), (60), (61), (62). It seems now well-established, by experiment, that rats, fed on a diet which is known to produce rickets, fail to develop the disease if they are exposed daily, for a short time, to sunlight or to the quartz mercury arc. A study of the chemical changes in the blood under sunlight treatment for rickets by Hess and Gutman (61), showed

that the inorganic phosphorus of the serum, which is reduced in children suffering from rickets (63) comes back to normal under sunlight treatment, or on the administration of cod liver-oil. These two agents seem to stimulate the deposition of inorganic salts and particularly affect the phosphorus metabolism of the body. These results are extremely interesting from a clinical standpoint and also give the first definite experimental evidence of metabolic change in the animal body brought about by sunlight.

5. *Malaria*. There is some evidence for believing that, in cases of chronic malaria, relapses may be induced by ultraviolet light (64), (65), (66). This is interesting in connection with the experiments by Levy (42) and Gassul (43) on the effect of ultraviolet light on the spleen. Work in this direction, however, has not progressed far enough to make any definite statements possible.

It has often been suggested that quinine owes its curative properties to its photodynamic action. This theory has never had any facts to substantiate it and has been thoroughly disproved by Sellards (49).

*Ecology*. There seems to be no doubt that, with extended knowledge as a result of more exact experiments, heliotherapy will be able to produce definite and valuable results. So far the experiments have seldom been with monochromatic light and little distinction has been made between the ultraviolet in sunlight and in artificial sources. As information on the light emission of different sources and the absorption of various materials is scattered and hard to find when needed, tables giving some of these facts have been put at the end of this paper (tables 6 and 7).

The study of the extent and intensity of the ultraviolet radiation in sunlight is of particular interest on account of its application both to heliotherapy and to ecology. In connection with his studies on the effect of the Philippine climate on man, Freer (67) determined the limit of the solar spectrum in the ultraviolet and found it to be  $291\mu\mu$ , which is identical with the limit as found at all latitudes and altitudes. Miethe and Lehman (68) made measurements at Assuan, Berlin, Zermatt, G6rnergrat and Monte Rosa and found the limit of the spectrum always the same,  $291.21$  to  $291.55\mu\mu$ . This limit, being fixed by ozone absorption in the outer atmosphere, is independent of any condition on the earth. However, the intensity of distribution in the solar spectrum, particularly in the ultraviolet end, is a different matter. Dorno (69), in a recent book, has summarized the findings on this subject and given the results of his own valuable work at Davos. He studied the daily

and yearly variations of the ultraviolet and blue in sunlight by means of photo-electric cells. Brightness determinations were made with a photometer, and bolometric and pyrheliometric determinations were made of solar heat. Most accurate heat measurements have been made by Abbot (70) and others but Dorno's work on the ultraviolet variation is unique. Figure 3 from his book shows the extraordinary variation in the intensity of ultraviolet radiation throughout the day, and also the

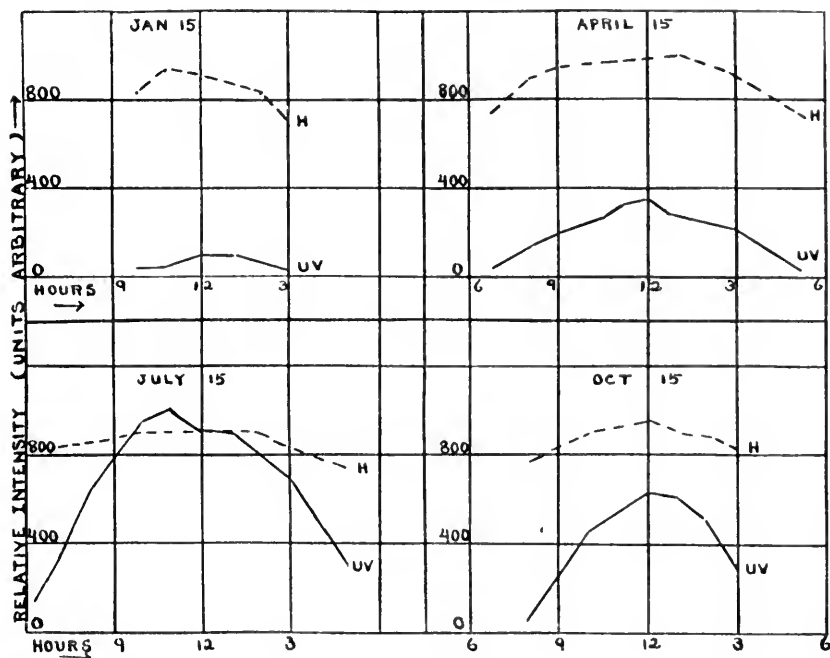


Fig. 3. Variation in intensity of solar heat,  $H$ , and ultraviolet solar radiation,  $UV$ , throughout the day, in different months. Experiments at Davos, Switzerland.

great difference between a day in summer and one in winter. Figure 4 also shows that the midday variation throughout the year is markedly greater for ultraviolet than for heat and visible light. Latitude and altitude must also make a great difference in ultraviolet intensity because with increase in altitude the transparency of the atmosphere increases faster for ultraviolet than for visible light. Since the ultraviolet light undoubtedly exerts an influence on the human organism, further studies in this field should yield most important ecological results.

Freer (67), Chamberlain (71) and others have carefully studied the effect of a tropical climate on the condition of soldiers in the Philippines, especially in reference to the effect on blondes. They found that all soldiers, irrespective of their coloring, reacted in the same way to life in the tropics. They showed loss in weight, higher pulse rate and rate of respiration, and a lower blood pressure. They had a high red count and low color index, and the differential white count showed a high percentage of lymphocytes and a low percentage of polynuclears (72).

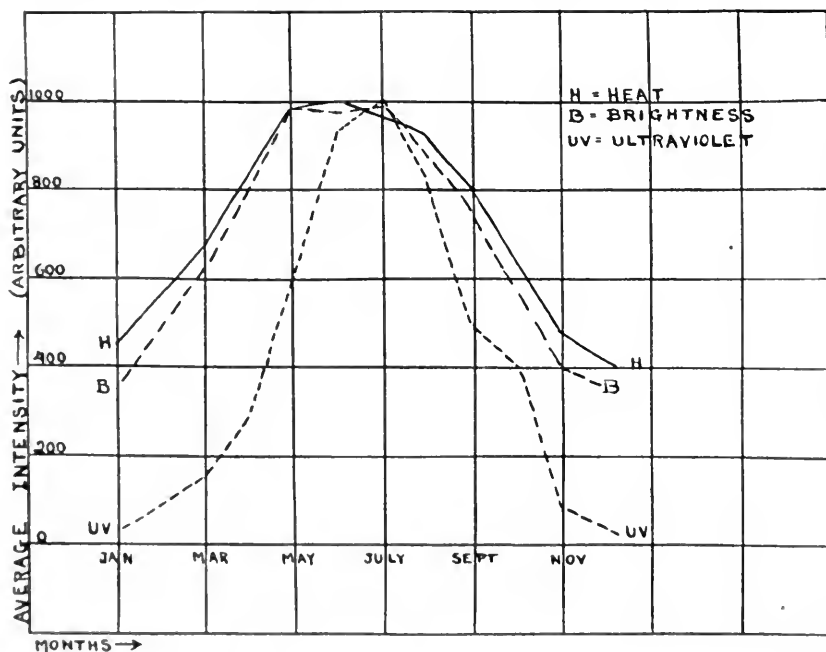


Fig. 4. Average midday intensity of solar heat,  $H$ , ultraviolet radiation,  $UV$ , and brightness,  $B$ , throughout the year at Davos, Switzerland.

The same changes in pulse, respiration, and blood pressure can be brought about by moist heat (73), and it is quite possible that the heat may also be responsible for the blood changes. On the whole, the work in the Philippines showed that the deleterious effects of the tropics are due to heat and not to light. Now in tuberculosis and rickets, and possibly in other diseases, light has a curative action, and the beneficial effects are probably located in the ultraviolet region less than  $330\mu$ . It seems then conceivable that the benefit we obtain from outdoor life

may be due, in part at least, to the ultraviolet of the sunlight. Indoor light is cut short at  $330\mu\mu$  by the absorption of glass.

*A theory of light action.* With our increasing knowledge of atomic structure, and the importance of the configuration of electrons in an atom in determining its physical and chemical properties, we are beginning to interpret chemical as well as physical reactions in terms of electronic behavior. In a general way it is safe to believe that the physiological effects of light have their origin in the photochemical reactions produced when light is absorbed. With simpler chemical substances light is seen to act as a powerful oxidizing and reducing agent, so we suppose that with the more complex chemical compounds in the living cell the same is true. Also, it is safe to suppose that all photochemical reactions are initiated by a change in configuration and velocity of the electrons of the substance absorbing the light. Light energy, absorbed by a body, may increase the molecular motion, thereby producing a rise in temperature. If the incident light has small enough wavelengths to produce vibrations in the electrons, instead of in the molecules and atoms, the absorption of light may result in the escape of electrons from the atoms, with a consequent change in valency, resulting in chemical action. When light of very short wavelengths (less than  $300\mu\mu$ ) falls on many substances, notably metals, where most of the energy is absorbed in the surface layer of atoms, the electrons absorbing this energy have their own energy increased to such an extent that they are shot off from the surface of the metal, leaving it positively charged. This phenomenon, discovered by Hallwachs in 1888, is called the photo-electric effect.

There is 1, the normal photo-electric effect, shown by a great number of substances, in which the number of electrons shot off increases with decreasing wavelength of the incident light; and 2, the selective effect, shown only by the alkali metals, in which a great number of electrons are shot out by light of a particular wavelength in a particular state of polarization (see fig. 5). This region of maximum activity may be at relatively long wavelengths; rubidium  $470\mu\mu$ , potassium  $435\mu\mu$ , sodium  $340\mu\mu$ , etc. Metals in general show photo-electric activity, their relative activity varying with surface conditions. Metallic compounds, especially sulphur and halogen compounds, show photo-electric action and are also extremely phosphorescent. Many non-metallic compounds, particularly many of the aniline dyes, are photo-electrically active. In metals, where absorption takes place in a surface film, electrons escape easily and a positive charge is left. This is also true of the photo-

electric dyes in a solid form. In liquids, where the penetration is deeper, the electrons do not escape so easily and besides the emission of electrons, there is often an increase in the electrical conductivity of the solution. This happens also in some solids, of which selenium is the most noteworthy example. The true photo-electric effect (i.e., escape

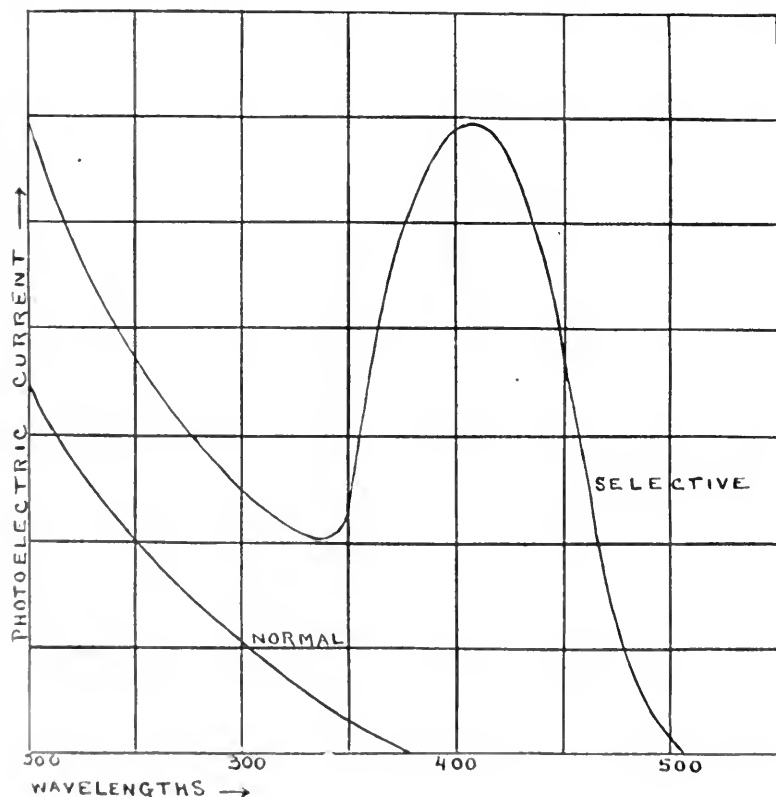


Fig. 5. Typical curves showing the normal and selective photo-electric effects.

of electrons from the surface) is usually stimulated only by far ultra-violet light, which is absorbed in a surface film. Inner photo-electricity, or ionization due to light, which shows as an increased conductivity, is due to light of longer wavelength which penetrates into the liquid or solid in question, and the region of maximum activity may lie in the visible or infrared.

The principal experimental results to be explained by theories of photo-electric action are these: *a*, uniform velocity of electrons for light of a given wavelength; *b*, velocity of electrons independent of intensity of light; *c*, number of electrons proportional to intensity of light; *d*, number and velocity of electrons increase with decreasing wavelength (i.e., increasing frequency) of exciting light; *e*, number and velocity of electrons independent of temperature; *f*, with increasing wavelength (decreasing frequency) the effect becomes 0 (i.e., there is a threshold frequency  $\nu_0$ , such that no light of frequency less than  $\nu_0$  causes emission of electrons and any light of frequency greater than  $\nu_0$  causes some emission.) Many physicists prefer to believe that light acts through resonance, the energy of the orbital motion of the electron being gradually increased by sympathetic light vibrations until the electron is able to escape from the control of the forces binding it to the atom. Usually, however, the photo-electric effect is interpreted in terms of the quantum theory. It is supposed that the kinetic energy of an electron is increased by the energy of one light unit =  $E$  = quantum of light energy =  $h\nu$ , where  $\nu$  is the frequency of the light and  $h$  is a universal constant. If this whole energy is given to one electron, and none is lost by subsequent collision, it leaves the body with energy =  $\frac{1}{2}mv^2 = h\nu - P$ , where  $P$  is energy lost in getting out of the atom. The energy, hence the velocity, of the emitted electron thus obviously increases with increasing frequency (decreasing wavelength). Suppose  $V = +$  potential just necessary to prevent the electron from leaving the body. Then  $Ve = \frac{1}{2}mv^2 = h\nu - P$ . The long wavelength limit of the photo-electric effect, or the photoelectric threshold, is that frequency ( $\nu_0$ ) for which the electron escapes with zero energy. It is therefore given by

$$0 = h\nu_0 - P$$

so that  $h\nu_0$  is the work required for an electron to escape from the atom and

$$Ve = \frac{1}{2}mv^2 = h(\nu - \nu_0)$$

This equation has been verified experimentally.

The existence of this threshold frequency ( $\nu_0$ ) is one of the most striking features of photo-electric action. It signalizes a sharp and absolute discontinuity in the phenomenon. It is a perfectly definite quantity when the condition of the body is definitely specified but it is extraordinarily sensitive to minute changes in surface conditions, such as is caused by the presence of extremely attenuated films of foreign matter. The importance of this change in threshold has only recently

been appreciated, and so far few experiments have been done on it. A much larger number of investigations has been carried out on the change in the total photo-electric current (photo-electric sensitivity to unresolved light) under different conditions. It is very probable that these changes are due to a shift in photo-electric threshold, a shift toward the red making a greater portion of the spectrum active and thereby increasing the total current. Pohl and Pringsheim (74) found that the photo-electric threshold of newly distilled calcium amalgam moved from 350 to  $600\mu\mu$ , and that of magnesium amalgam from 350 to  $550\mu\mu$  in 24 hours, without apparent cause. In the near future much more information on this important point will undoubtedly be available.

Throughout this paper, and throughout all the literature on light action, there has been continual reference to the phenomenon of fluorescence. This term is often vaguely understood. The term luminescence is a convenient one to include all cases in which there is an emission of light. We distinguish between electroluminescence, due to such agencies as cathode rays; triboluminescence, due to friction and crushing; chemiluminescence, due to chemical action (to this type belongs the bioluminescence of fireflies and sea animals, which latter is unfortunately spoken of usually as phosphorescence); and photoluminescence, due to light.

In electroluminescence we can see that the excitation must be closely connected with the displacement or separation of electrons from the atoms of the substance. In triboluminescence, bearing in mind the facts of frictional electricity, it is also probable that displacement or separation of electrons must take place. In chemiluminescence we have the rupture of a chemical bond (displacement of a valency electron). So when we come to photoluminescence it is natural to suppose that the separation, either partial or complete, of electrons from the atom is an important stage in the process by which emission of light is brought about.

Photoluminescence includes both fluorescence and phosphorescence. In both cases light of one wavelength, falling on a substance, results in the emission of light of another and longer wavelength. For instance, quinine shines with visible blue light when illuminated by ultraviolet. Luminescence observed only while the exciting light acts is called fluorescence, and that which continues after the stimulus ceases is phosphorescence. Generally speaking, liquids and vapors are fluorescent, and solids show both fluorescence and phosphorescence. Lenard and Saeland (75) have shown that phosphorescent solids are usually photo-

electric and Stark and Steubing (76), in studying organic compounds, showed that those which fluoresce easily are generally photo-electrically active in a solid form. This is not easily observed in solutions since the emitted electrons are caught by the molecules of the solvent and do not escape, and the photo-electric effect becomes latent (77).

When exciting light falls on a fluorescent substance we may suppose that electrons are separated, partially or completely, from their parent atoms, but that this separation represents an unstable condition and recombination takes place with the emission of light. In the case of phosphorescence, a finite time elapses between the separation of the valency electrons and their return. Although fluorescence in general is accompanied by photo-electric action, this is not essential since the separation of the electrons may be only partial. We therefore can regard fluorescence and phosphorescence as evidences of a complete, or incomplete, separation of electrons under the action of light, with a subsequent return to the atom.

From the point of view of modern physics, we may regard it as practically certain that the first stage in any photochemical reaction consists in the separation of valency electrons under the influence of light. In the true photo-electric effect the electrons are lost from the substance, in fluorescence they return to their original atom, in photochemical reactions they attach themselves to some other atom, or group of atoms. In general, maximum fluorescence is given by wavelengths too long to produce any photo-electric action at all. In other words, under visible light the energy absorbed by an electron ( $h\nu$ ) is too small to separate it completely from the atom. Pauli (78) studied the ratio of the intensity of fluorescent light to exciting light, and also the degree of photo-electric action, as a function of the wavelength of the exciting light. In the substances used, maximum fluorescence was stimulated by visible light from 400 to 500 $\mu\mu$ , whereas the photo-electric effect began in the ultraviolet and increased with decreasing wavelength. The two effects, however, overlapped over a considerable range. Thus, in the presence of very great photo-electricity, there would be little chance of fluorescence and photochemical action would be confined to a surface film. In cases of very strong fluorescence, with only a partial separation of electrons and a swift return to the original atom, there would be few electrons lost or joined to new atoms, and therefore little photo-electric or photochemical action. But at intermediate stages all three might occur simultaneously, and constitute three different possible manifestations of the displacement of valency electrons as the result of the absorption of light energy.

One photochemical reaction, which is universally familiar and of special use as an analogy in physiological actions, is the formation of an image on a photographic plate. Joly (79) is responsible for an explanation of this reaction which is a necessary step in the development of this theory. He advanced the theory that "the beginnings of photographic action involve an electronic discharge from the light sensitive silver halide molecule. In other words, the latent image is built up of ionized atoms or molecules upon which the chemical effects of the developer are subsequently directed." In support of this it is known that silver halides are vigorously photo-electric, with their activity in the descending order, bromide, chloride, iodide (the same order as their photographic sensitiveness). Also photographic images are produced by x-rays and radium, which are strong ionizing agents. It may be that the ionization brings about a chemical change and that the chemical product is the latent image, but as the latent image can be formed at temperatures approaching absolute zero, this explanation is not likely. If a gel containing silver halide grains is illuminated, the photo-electrons emitted move out with velocities depending on the wavelength of the light and, when illumination ceases, there is a charged grain surrounded by gel in which negative electrons are disseminated, the radius of the sphere of distribution depending on the velocity of the electrons emitted. The developer then reacts chemically with this latent image to effect reduction of the silver halide grains. On this theory a very complete explanation of photographic reversal is possible (80).

To this theory, as given by Joly, the following suggestion should be added which, so far as the author knows, has not been given before. An unsensitized plate responds only to short wavelengths, which is to be expected because, except for the selective effect, photo-electric action is given only by short wavelengths. After sensitization, plates respond to longer wavelengths. Since sensitizers are many of them known to be photo-electric it seemed at first that they might possess selective photo-electricity in the region of their absorption bands, thus making the plates photo-electrically active at longer wavelengths. But since the photo-electric activity of the sensitizers so far investigated is not of the selective type, this is not supported. It seems more likely that the sensitizers act by shifting the photo-electric threshold of the active silver halide particles to longer wavelengths so that they become photo-electrically active and capable of forming a latent image throughout the visible, and even into the infrared.

If we now consider the physiological actions of light on this theory we see that they fall in line very easily. For light of wavelength less than

300 $\mu$  we get intense reactions, due primarily we may suppose to the ionization of photo-active elements in the skin and blood of higher animals, and in the protoplasm of lower animals. This is analogous to the formation of the latent image in photography. Subsequent chemical action on these ionized substances produces changes which, in certain cells, result in the formation of the pigment melanin. In the particular case of pigment formation one might guess that tyrosin was the photo-active element involved. Besides pigment formation in the basal epithelial cells there are undoubtedly many other photochemical reactions induced by the ionized condition due to light. Ordinarily, light of longer wavelength is ineffective in producing physiological changes but the living cell, like the photographic plate, can be sensitized. In the presence of certain substances, such as cosin and hematoporphyrin, the photo-electric threshold of the cell, or of some of its constituents, shifts towards longer wavelengths and we get the same chemical changes initiated by visible light that we usually get only with ultraviolet. These changes naturally take place at the absorption bands of the sensitizer where the greatest amount of light energy is absorbed and made available for transformation into electronic energy. This shift in photo-electric threshold is easily brought about *in vitro*, giving rise to the many results obtained in photodynamic investigations. *In vivo* it happens only rarely, hematoporphyrin alone being capable of creating any marked sensitivity to visible light. There is considerable evidence to show that diseased tissue is more susceptible to radiations than normal tissue. To push the photographic analogy further, we may assume that normal tissue acts like a slow plate, and diseased tissue like a fast one.

In the green plant one may suppose that the chlorophyll produces a sensitized condition to visible light and makes it normally photo-active to long wavelengths, at the same time giving greater absorption in the visible. An analogy between chlorophyll and the sensitizers of a photographic plate has frequently been drawn (81). If, as has been postulated, the sensitizer acts by shifting the threshold toward the red, as well as by increasing the absorption in the visible, the plants without chlorophyll should show photo-synthesis in the ultraviolet. Experiments by Stoklasa (82) show that, in etiolated plants, the formation of chlorophyll proceeds at a greater rate under the quartz mercury arc than in sunlight. So it seems probable that the early stages of photo-synthesis proceed faster in ultraviolet light and result in the formation of chlorophyll which then protects the plant against an overdose of ultraviolet, and sensitizes it so that synthesis then proceeds in the visible.

Reasoning along this line leads one to suppose that the pigment in human skin may act in a somewhat similar way, forming under ultraviolet light, and then acting as a sensitizer so that wavelengths greater than  $300\mu\mu$  become active in producing physiological effects. If this were true one would expect the good results of heliotherapy only after pigmentation had taken place. If, on the contrary, the chemical action resulting in the formation of pigment is the principal source of benefit, one would expect good results chiefly during the period of pigment formation.

The theory stated above can be summarized as follows. Light shorter than  $300\mu\mu$  acts on the living cell by ionizing its photo-electric constituents, and thereby leading to photochemical action. Light longer than  $300\mu\mu$  acts in the same way in the presence of sensitizers, which so affect the surface conditions of these constituents that their photo-electric threshold is shifted into the visible, and they therefore become ionized, with resulting chemical action, when illuminated by visible or near ultraviolet light.

This theory is based on purely theoretical considerations, but a recent paper by Schanz (83), written in support of a different view, contains experimental results which, although in no sense a proof, give more support to the theory here described than to that adopted by him. Schanz found that egg albumin is photo-electric and then examined its activity when combined with various photo-electric fluorescent dyes some of which are sensitizers and some not. Some of his results are given in table 5.

Schanz's explanation is that the sensitizers emit electrons which, by ionizing the molecules of the egg albumin, effect in it a chemical change. However, as none of the fluorescent substances used show any photo-electric effect for light through glass, there is no reason to suppose that they would by their own photo-electricity sensitize to visible light. If, however, the combination of egg albumin and a sensitizing dye resulted in shifting the photo-electric threshold of either toward the red, with resulting photo-electric action for wavelengths greater than  $330\mu\mu$ , there would then be reason to expect photo-activity in the visible and near ultraviolet. The fact that Schanz found eosin and egg albumin together more photo-electric than either alone makes it probable that this actually occurred. Schanz however did not investigate the long wavelength limit of the effect and experiments are now in progress in this laboratory to test this point and to see whether, in general, substances sensitized so as to react in visible light, also show a shift in photo-electric threshold.

A number of scattered results give considerable evidence in favor of a photo-electric theory of photosynthesis. Becquerel (84) exposed collodion films containing silver bromide and chlorophyll to the solar spectrum and found reduction of the silver bromide in bands corresponding to the absorption bands of chlorophyll, and therefore to the region of photosynthesis. Dixon and Poole (85) found no photo-electric action in visible light in acetone extracts of pulverized leaves, as measured by the discharge of electrons from the surface. On the other hand Waller (86), Ries (87) and Samsonov (88) have all found that the Becquerel effect (production of a current by illumination of one electrode) was very marked when chlorophyll was used as the electrolyte. The maximum action in visible light was in the red, and

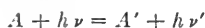
TABLE 5

SUBSTANCE	LEAK OF ELECTROMETER IN 3 MINUTES
	<i>Divisions</i>
Fuchsin + H <sub>2</sub> O.....	100.00
Fuchsin and egg albumin.....	40.00
Methylene blue + H <sub>2</sub> O.....	100.00
Methylene blue and egg albumin.....	7.70
Fluorescein + H <sub>2</sub> O.....	2.86
Fluorescein and egg albumin.....	2.63
Eosin + H <sub>2</sub> O.....	1.18
Eosin and egg albumin.....	6.06
Egg albumin + H <sub>2</sub> O.....	1.85

there was no effect in the green, again corresponding to the region of photosynthesis. This tends, indirectly to support the general theory of light action given above.

The importance of light in initiating chemical and physiological reactions has not been generally recognized in the past and perhaps should not be stressed too much now, without further experimental facts to substantiate it. But in closing, reference may be made to a remarkable paper recently published by Perrin (89) in which he boldly makes the hypothesis that all chemical reaction is initiated by the absorption of radiation (heat, light, etc.), and that the speed of the reaction is deter-

mined by the intensity of this radiation. He represents all chemical changes by equations of the type



Matter in a state of stable equilibrium  $A$ , on the absorption of a quantum of energy  $h\nu$ , goes into a state of equilibrium  $A'$  with the emission of a quantum of energy  $h\nu'$ , and vice versa for a reaction of a reversible type;  $h(\nu - \nu')$  represents the heat of reaction, and for the particular reaction  $3\text{O}_2 \rightleftharpoons 2\text{O}_3$ , which he considers in detail, where  $\nu$  and  $\nu'$  are the ultraviolet absorption frequencies of oxygen and ozone (wavelengths  $165\mu\mu$  and  $260\mu\mu$  respectively) the heat of reaction is actually closely represented by  $h(\nu - \nu')$ . On this theory all chemical change  $A \rightarrow A'$  is initiated by the absorption of radiation of frequency  $\nu$  and proceeds with the emission of fluorescent radiation of frequency  $\nu'$ , which may be in the infrared and hence invisible. This is only a hypothesis as yet but it leads to a satisfactory explanation of certain facts about the velocity of chemical reactions which were not explained on the older kinetic theory. Recent calculations by Langmuir (90) show that Perrin's theory does not apply to many cases of molecular dissociation in the simple form given above but suggest an additional source of energy for which the radiation may act as a releasing trigger. There is, however, in the paper a suggestion that photo-electric action in an enlarged sense may be one of the most fundamental and important occurrences in nature.

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TABLE 6

*Emission spectra of various sources in the visible and ultraviolet*

Sunlight.....	Continuous spectrum, except for the absorption of the Fraunhofer lines. Short wavelength limit = $291\mu$ , due to the absorption of ozone in the upper atmosphere.
Quartz mercury arc .....	Discontinuous spectrum giving a number of bright lines from $230\mu$ to $579\mu$ . There are a number of weak lines below 230, the shortest being 185. The arc itself emits shorter lines which are absorbed by the quartz.
Electric sparks.....	Discontinuous. Many sparks give lines of shorter wavelength and greater intensity than the mercury arc. The aluminum spark gives strong lines at 185, 186, 193 and $199\mu$ .
Hydrogen discharge tube	Discontinuous. Relatively feeble in the visible but rich in very short wavelengths. If filtered through fluorite there are a large number of lines between 103 and $165\mu$ .
Carbon arc.....	Band spectrum, rich in near ultraviolet but weak beyond $300\mu$ , practically nothing below $240\mu$ .
Iron arc.....	Very rich line spectrum with lines so close that it amounts almost to a continuous spectrum as far as $230\mu$ .
Tungsten arc.....	Richer in lines than the iron arc, especially good in the ultraviolet as far as $210\mu$ .

TABLE 7

*Transparency limits in the ultraviolet*

SUBSTANCE	LIMIT OF TRANSMISSION
	$\mu$
Fluorite (1-2 mm. thick).....	125
Crystalline quartz (0.2 mm. thick).....	145
Crystalline quartz (2.0 mm. thick).....	150
Rock salt (2 mm.).....	177
Clear quartz glass (fused silica).....	180
Uviol glass.....	280
Common soda glass (0.2 mm.).....	300
Common soda glass (2.0 mm.).....	330
Water.....	190
Air.....	180

# THE MECHANISM OF MUSCULAR CONTRACTION

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In 1914, the present writer prepared a review (33), for the *Ergebnisse der Physiologie*, on the relation between the heat-production of muscles and the chemical processes taking place in them. An account and bibliography, up to that date, of the work relevant to the present subject was given there, and it not proposed to cover this ground again, except insofar as more recent investigations require a revision of, or reference to, the earlier work. Neither is it proposed to discuss in general the theory of muscular contraction; the following pages are confined rather to an account of the various physical, chemical and mechanical phenomena exhibited by the active muscle, together with a speculation on their immediate causes. At present the phenomena themselves are complex enough, and ill-understood, and until we have an adequate foundation of fact it is worse than useless to erect an edifice of theory which will certainly topple down, as all theories of contraction have done before.

PART I. MECHANICAL PHENOMENA. Evidence was given by A. V. Hill (28), (31), and by Hartree and A. V. Hill (23) (24), that it is simpler to regard the potential energy set free, rather the actual work done, as the mechanical end-product of physiological activity in muscle. This potential energy, which we will term the "theroretical maximum work,"  $W_0$ , is widely different from  $W$ , the work actually realisable in practice. The difference is due to several factors: *a*, to the conditions of loading—if the load be too heavy the muscle cannot move it, and no work will be done; if it be too light the muscle cannot exert its full strength, and the work will be reduced; and between these limits every value of the load tends to waste some of the theroretical maximum work,  $W_0$ ; *b*, to the fact that (in a twitch or a short tetanic contraction) relaxation may have commenced before the shortening is complete; and *c*, to the viscous resistance of the muscle to a rapid change of form. The actual work  $W$  is determined therefore by the manner and speed with which the shortening is carried out, while the theroretical maximum work  $W_0$  depends only on the stimulus, and on the condition,

temperature, and length of the muscle fibers. Hence, in comparing the mechanical with the thermal (or the chemical) response of muscle, it is desirable to employ the theoretical maximum work  $W_0$  as the basis of comparison.

*The theoretical maximum work.* If the force exerted by a stretched elastic body be plotted against the distance through which it has been stretched, we obtain a "stress-strain diagram," the area of which gives the amount of work expended in the stretching. If the elastic body be one which does not show the phenomena of "after-extension," i.e., does not continue to strain when a constant stress is applied, the same amount of work can be recovered by allowing the body to shorten again. The theoretical maximum work  $W_0$ , or the potential energy, of the stretched elastic body, is then equal to the area of the stress-strain curve, and can be determined by experiment and calculation. If however the body, like rubber, be one showing the phenomena of after-extension, the work done in stretching will be equal to the work recoverable in shortening *only if both processes be carried out indefinitely slowly*. If either process be carried out at a finite speed, a finite proportion of the work, or of the potential energy, will be dissipated irreversibly by internal friction as heat. Muscle shows the phenomena of after-extension in an exaggerated form (see Hartree and A. V. Hill, (27)).

Suppose that a muscle be subjected to a stimulus of known strength and duration, and allowed to shorten freely to a length  $x$ , after which it comes up against a dynamometer of some kind, and the force  $f$  which it can exert isometrically at that length is measured. We thus obtain a curve relating force to length, and the area of this curve gives the theoretical maximum work  $W_0$  of the excited muscle (31, p. 450; 56, p. 145). From this must be subtracted the work done in giving the muscle its initial extension, if any. The precise details of such a determination of  $W_0$  are still a matter of dispute (see e.g., Meyerhof (56)), and no final conclusion can be said yet to have been reached: it is clear, however, that we are on the right road in seeking for an expression of some kind for the maximum work theoretically available in muscular activity. In any actual shortening, either of an active muscle or of a muscle passively stretched, the amount of work obtainable is less than this theoretical maximum, owing to the fact that the muscle can exert its maximum force at any length only if it be given a considerable time at that length to develop it. This is due to the same cause as the phenomena of after-extension in muscle, viz., to the viscous resistance of the muscle-substance to a rapid change of form. (See fig. 1.) This

phenomenon of after-extension is presumably due to the fact that muscle consists of a fine elastic network, containing a viscous fluid. Any change of shape necessitates a flow of fluid to a new position, and if a force be applied to the muscle it will be used, partly in stretching the network, and partly in driving the viscous fluid of the muscle through it.

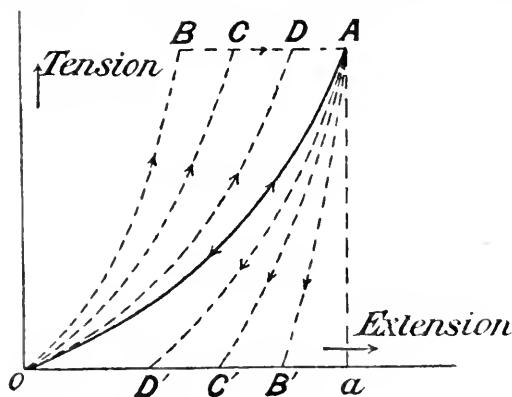


Fig. 1. Relation between tension and extension in a muscle. The full curve  $OA$  corresponds to a very slow process of unloading or loading the muscle from or to a given tension, and represents a "reversible" process. The broken curves represent "irreversible" processes carried out more or less rapidly. The curves  $OBA$ ,  $OCA$  and  $ODA$  correspond to *loading* carried out rapidly, the most rapid being  $OBA$ , and the least rapid  $ODA$ . The curves  $AB'O$ ,  $AC'O$ ,  $AD'O$ , correspond to unloading carried out rapidly, the first being the most and the last being the least rapid. The potential energy possessed by the stretched muscle corresponds to the area  $OAAa$ : the work done in stretching it rapidly along (say) the curve  $OCA$  corresponds to the area  $OCAAa$ : the work obtained from it on unloading it rapidly corresponds (say) to the area  $AC'a$ : the work lost and degenerated into heat irreversibly in the complete cycle corresponds therefore to the area  $OCAC'$ .

(Note.—The curves are illustrative only and do not represent an actual observation.)

*The realisable work.* Most modes of contraction are inefficient. Either *a*, the load is too great and the muscle cannot finish its shortening; or *b*, the load is too small and the muscle cannot exert its full force; or *c*, relaxation has set in before the shortening is complete. In each case only a fraction of the available potential energy is realised as work: the rest is wasted as heat in the muscle. It is possible completely to avoid losses due to *a* and *b* by opposing the contractile force of the muscle, not to a load but to the inertial reaction of a mass: in this case the "reaction" of the mass adjusts itself exactly, neither more nor less,

to the "action" exerted on it by the muscle, and the potential energy of the muscle is free to be transformed into the kinetic energy of the mass. By varying the size of the mass the time occupied in the shortening may be varied, and so adjusted that the shortening is complete before relaxation has commenced. In this way we may avoid all losses due to the factors  $a$ ,  $b$ , and  $c$  above. There remains however one loss which we cannot eliminate, that due to the internal friction of the muscle substance itself.

Poiseuille's formula for the flow of a viscous fluid in a capillary tube states that the volume of fluid driven through per second is proportional to the pressure: in other words, the work done (and dissipated as heat) in driving a *given quantity* through is proportional to the speed with which the process is carried out. Turning to the case of a muscle, if the mass against whose reaction it pulls be large, and if its contraction be maintained, its speed of shortening will be small, and little of its potential energy will be wasted in overcoming its own viscous resistance to a change of form. Under such conditions the kinetic energy produced should approximate to the theoretical maximum work  $W_0$ . If, however, the shortening has to occur within a limited space of time, as e.g., in a twitch, the mass cannot be made very large, and the force exerted by the muscle at any length will be balanced, partly by the reaction of the mass, partly by the frictional resistance of the muscle substance itself to a rapid change of form. The latter factor will cause  $W$ , the actual work done, to be appreciably less than  $W_0$ , the elastic potential energy, the difference,  $(W_0 - W)$ , being dissipated as heat within the substance of the muscle. This has been shown in two ways:  $a$ , by Hartree and A. V. Hill (27, p. 165) on isolated muscles passively extended, the more rapid the shortening the less being the external work done: and  $b$ , by A. V. Hill (35) on human muscles, the proportion wasted, viz.,  $\frac{(W_0 - W)}{W_0}$

of the total potential energy developed, increasing with the speed of shortening according to the formula:

$$\frac{(W_0 - W)}{W_0} = \frac{k}{t}$$

where  $k$  is a constant depending on the viscosity of the muscle fluids, and  $t$  is the time occupied in the shortening. The work done can be expressed therefore in the form

$$W = W_0 \left(1 - \frac{k}{t}\right)$$

This expression is very accurately true for the case of human arm-muscles.

The muscle twitch, especially at higher temperatures, lasts only a little time, and if shortening is to be complete before relaxation has commenced it has necessarily to occur rapidly. Consequently a considerable proportion of the theoretical maximum work has to be dissipated as heat. An investigation has been made by Doi (11), employing an inertia device (34) for measuring the maximum work, of the quantity  $\frac{W}{Tl}$ ,  $W$  being the work realised in a maximal twitch,  $T$  the force developed in a maximal isometric twitch, and  $l$  the unextended length of the muscle. The value found was about 0.04 at 15°C., and was practically independent of the degree of extension. The calculation of the potential energy of the excited muscle, from the tension-length diagram (31, p. 453), gives a value for  $\frac{W_0}{Tl}$  of about 0.14: hence in a twitch at 15° the

realisable work  $W$  is only about  $\frac{1}{3}$  to  $\frac{1}{4}$  of  $W_0$ , the potential energy. Meyerhof (56, p. 154), employing analogous means, finds that the work in a twitch is always considerably less than that calculated from the diagram, especially in cases (e.g., rapid twitches at a high temperature) where theory requires a greater frictional degradation of energy. In prolonged contractions, on the other hand, he has shown that the work realised approximates to that calculated from the diagram. If the potential energy be not used up completely, either in doing external work, or in the irreversible viscous processes associated with the change of form of the muscle, in other words, if shortening be not complete when relaxation sets in, then what is left of the potential energy must somehow be dissipated as heat in relaxation. Hartree and A. V. Hill (23) have shown that there is a considerable evolution of heat during relaxation following an isometric contraction, and this would seem to represent—at any rate in part—the heat-equivalent of the potential energy developed during contraction.

If a muscle be given an unsuitable load it will waste its mechanical potential energy in one of two ways: if its load be too small it will move too fast and so dissipate too great a fraction of its potential energy in viscous processes inside it; if its load be too great it will move too slowly, or not at all, and will dissipate too great a fraction of its potential energy in relaxation. Thus there are really only *two* processes by which the theoretical maximum work  $W_0$  can be wasted as heat, viz.: *a*, frictional loss, due to the rapid change of form; and *b*, relaxation. In any actual contraction both factors play their part.

*Work collectors.* Of such instruments Fick's "Arbeitsammmler" (17, p. 140) is perhaps the best known. The same author described a "Winkelhebel" (17, p.55), and a "Schwunghebel" (17, p. 63) the principles of which have been used by Meyerhof (56). A. V. Hill (34), (27), (11) has described an inertia lever balanced on knife edges, for use with isolated muscles, and (35) a flywheel device for use with human muscles. All these instruments agree in principle in attempting to oppose the contraction of the muscle, at every stage, by a force just equal to the tension it can exert. In order to secure the greatest amount of work from a contracting muscle this principle is necessary, and should be embodied in any ergometer by the use of a suitable inertia, or otherwise. In the case of human muscular movements the conditions are apt to be somewhat altered by the fact that the mechanical "gearing-up" of the muscles, by their attachments in the body, may decrease as shortening proceeds, a fact which may make an isotonic contraction much more efficient than it is with an isolated muscle. The subject however of mechanical efficiency is dealt with below.

*The isometric contraction.* The actual shortening of a muscle is a complex process, involving a variety of physical and mechanical factors, in addition to the purely physiological ones concerned with the extent and course of the response. In order to eliminate these extraneous factors it is advisable, where possible, to deal with rigidly isometric contractions. Physiological literature is burdened with observations which depend rather on the properties of levers than on those of muscles, and it is possible to avoid any mechanical complication by employing an isometric spring-myograph, with good sensitivity and high natural frequency. (See (23 p. 115), (65, p. 245), (10).)

*The effects of temperature.* The rate of development of the isometric twitch has a high temperature coefficient, viz., about 2.5 for 10°C., and its rate of disappearance a still higher one, about 3.6 (25). These point to chemical reactions as the basis both of contraction and of relaxation. Theories of muscular activity have tended to neglect relaxation, or to minimise its importance, attributing it in some vague way merely to a reversal of contraction. Its high temperature coefficient, however, and other more direct evidence, force us (see p. 320 below) to regard it as a positive process, due to its own special chemical reactions, and in no way subsidiary to the other processes of contraction.

A rise of temperature affects not only the time-relations of a twitch but also its absolute size. At any but extreme initial extensions (10) the effect of a rise of temperature is to diminish the size of the twitch,

in the same way as it diminishes its work (11), or its heat-production (12), (24). This probably arises from the fact that the amount of energy liberated in a twitch depends upon the *duration* of the change evoked by excitation, this duration being less at a higher temperature.

*Initial extension.* It was shown by Evans and A. V. Hill (16) that the force developed in an isometric twitch rises at first as the initial extension of the muscle is increased, reaches a maximum, and then rapidly decreases. The same is true of the heat-production. These experiments have been confirmed and extended by Doi (10), (11), (12), who has shown moreover that the maximum work exhibits the same dependence on the initial length of the muscle. Similar relations are true of the heart-beat, reading "pressure" for "force," "filling" for "extension," and "oxygen-intake" for "heat-production" ((10, p. 224); (37); (62); (47), (48); (60)). The speed also at which relaxation occurs is considerably decreased by an initial extension of the muscle (25), a fact which again is true of the heart (22). Finally, the temperature coefficients (25) of various factors in the twitch are affected by extension of the muscle. Of these phenomena, which are clearly of a very fundamental nature, no explanation can as yet be given: an extension of the muscle fiber from its natural resting length obviously modifies the surfaces or media in which the active processes of contraction and relaxation occur. The facts appear to be of great importance in relation to the heart.

*Strength of stimulus.* The time-course of the isometric twitch of a muscle directly stimulated is affected by strength of shock. The response to a weaker shock is invariably more prolonged than that to a stronger one, a fact difficult to explain on the "all or none" theory (25). (See p. 323 below.)

*Recovery of contractility.* The return of contractile power following a twitch has recently been studied by Adrian (1) and by Hartree and A. V. Hill (25). This "recovery" of contractility has nothing to do with the oxidative recovery process discussed below. If a muscle be subjected directly to two maximal shocks in succession, at a known interval, the effect of the second shock can be determined by comparing the response to the first shock only, with the combined response. Adrian found, in the case of cardiac muscle, that after a shock there is an absolute refractory period during which no response is given to the second shock, but that as the interval between the shocks is increased the response to the second shock increases to its original value, passing in the case of hearts perfused with an acid fluid, through a phase of super-

normal contractile power, before settling down to its original state. In the case of skeletal muscle the time-relations are very different, and Hartree and A. V. Hill were able to demonstrate the same recovery of contractile power only by subtracting from the ordinates of the combined curve the ordinates of the curve produced by a single shock. If this be done it will be seen that, following a shock, the contractile power is at first nil, then rises to its original value again, passing invariably, however, in the case of skeletal muscle, through the supernormal phase. The ordinary so-called "beneficial effects of contraction" are entirely artificial, and due (1, p. 16) to the onset of fatigue and to recording instruments with too much inertia.

*Tetanic contraction.* The prolonged contraction is built up as the resultant of the responses to the individual shocks. The manner in which this building-up occurs has been discussed by Hartree and A. V. Hill (25), who find that successive elements of the response evoked by successive units of the stimulus differ from one another, becoming smaller and more prolonged as the stimulus proceeds, until finally a steady state is reached. The properties of the prolonged contraction are similar, in many ways, to those of the twitch. The effects of temperature on the time-course of the contraction are the same; on the size of the response, however, they are in striking contrast, a rise of temperature slightly decreasing the size of the twitch, but considerably increasing that of the prolonged contraction. The explanation of the latter effect is simple: the recovery of contractility following a twitch (see above) is much more rapid at a higher temperature, so that each element in the response is much less reduced by its predecessors. This factor more than counterbalances the slight diminution of the response to a single shock.

PART II. THE HEAT-PRODUCTION OF MUSCLES: *Methods.* There are two means of measuring directly the liberation of heat in muscles; *a*, the calorimetrical method for cases of prolonged heat-production; and *b*, the thermopile, for cases where the tissue is small and the heat-production of comparatively short duration.

The *calorimetrical* method involves the use of a good non-conducting container, such as a Dewar flask, and either a differential arrangement (29), or an accurately maintained thermostat (53, p. 258), in order to control loss of heat by conduction, etc. This method is suitable for the investigation of the heat liberated during prolonged stimulation, during survival with or without oxygen, during the onset of rigor, or during prolonged oxidative recovery. (See (29); (61); (53), (54); (58).) In

order to quicken the oxidative recovery process Parnas used a cylindrical brass container, within which muscles could be kept in 2 or 3 atmospheres of oxygen, the container being placed in water in the calorimeter: a similar instrument was used by Meyerhof (54).

The *thermopile* is suitable for the investigation of the total heat produced in a contraction, of the time-relations of the heat-production, either "initial" or "recovery," and of the thermal changes associated with the passive lengthening or shortening of the muscle. In principle its use consists merely in placing a muscle in contact with the junctions of a thermopile, and recording the deflection of a sensitive galvanometer resulting from the rise of temperature. In practice a variety of precautions must be taken. In particular it is necessary to avoid differences of temperature at different parts of the instrument, or a gradual change of temperature of the whole instrument which will inevitably cause such differences: otherwise it is impossible to ensure a stable zero, or to make observations on muscles allowed to shorten (shortening may lead to warmer, or colder, points of the muscle coming on the junctions). Such thermopiles have been discussed by A. V. Hill (33) and by Bürker (6); recently Hartree and A. V. Hill (23), (26) have described modifications by which the zero may be maintained over a long period, the muscle subjected to any desired medium and allowed to shorten if required, photographic recording adopted, and quick and accurate calibration ensured.

As regards indirect methods of measuring the heat-production, apart from respiratory experiments applied to the whole animal, the only important method would appear to be the Warburg-Siebeck method, as employed by Meyerhof (54, p. 288), by means of which the oxygen absorbed by a single gastrocnemius of a frog may be accurately determined.

*Thermo-elastic properties of muscle.* According to Le Chatelier's rule, and to the Second Law of Thermodynamics, an elastic body suddenly subjected to a stress should show a thermal effect tending to neutralise the result of the stress. For example, if a steel wire be loaded suddenly its temperature will fall, because a fall of temperature causes the wire to shorten, and so tends to neutralise the lengthening produced by the load. These thermo-elastic effects are by no means small: they may easily be observed, and even employed in calculating the stresses in structures suddenly loaded (9). Realising that in the case of muscles allowed to shorten these thermo-elastic phenomena must necessarily be superimposed upon the other thermal effects accompanying contrac-

tion, Hartree and A. V. Hill (27) investigated them experimentally in living muscle, in dead muscle, and in india-rubber. These substances, unlike most others, shorten on being warmed, so that extension should lead to a rise, and shortening to a fall of temperature. As seen in figure 2, extension of a muscle leads indeed to a rise of temperature, as predicted, while shortening leads at first to a fall, which fall however is rapidly absorbed in a subsequent rise. These phenomena were found to be due to two distinct processes, a reversible and an irreversible one. The reversible one is that predicted from thermodynamical theory and discussed above. The irreversible one is due to heat produced at the expense of mechanical energy, by viscous resistance to the change of

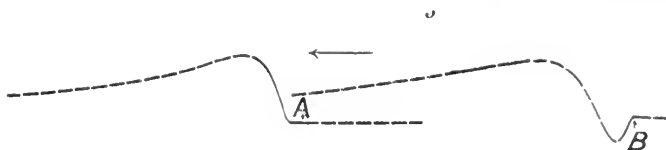


Fig. 2. Pair of sartorius muscles from *Rana temporaria*. Permanent load 5 grams. At A on the left hand curve 155 grams were hung gently on the muscle: the temperature rose. At B on the right hand curve, after the 155 grams had been hanging on the muscle for some time, the load was gently removed: the temperature fell rapidly (the reversible effect) and then rose (the irreversible "viscous" effect) and finally fell again (the physical loss of heat by conduction). Time in seconds shown as gaps in the curves. Read from right to left.

form of the muscle (see p. 313). The viscous effect increases the thermo-elastic effect in the case of extension, and decreases it in the case of shortening.

These experiments, which were repeated under various conditions, confirm the conclusion that the external work done in a muscular contraction is diminished through viscosity by an amount depending upon the velocity of shortening (p. 313 above). Further, they are a necessary preliminary to certain investigations which have never yet been satisfactorily made, but for which the means are now available, and which are of the most fundamental interest; viz., those in which the muscle is allowed to shorten during the contraction, the heat-production being compared with the work directly measured, and the effect of the shortening on the total energy liberated being determined.

*The "initial" heat-production.* Under ordinary conditions, and provided that the time of stimulation be not too long, the maximum deflection of the galvanometer is an accurate measure of the total heat given out in the development, maintenance and disappearance of the mechani-

cal response, i.e., in all phases except recovery. We shall refer to this heat as the "initial" heat. It was shown by Weizsäcker (67), (68) that the magnitude of the initial heat is uninfluenced by the presence or absence of oxygen, and by Hartree and A. V. Hill (23), employing a very sensitive method of comparison, that the time-relations of the initial heat are also quite uninfluenced by oxygen. Thus the "initial" breakdowns are entirely non-oxidative in character. It is clearly necessary to attribute two distinct mechanisms to the muscle, one similar to an electrical accumulator with an electromagnet and the necessary key and wires, by means of which chemical energy previously stored can be used either to do external work or to maintain a force as and when required, the other similar to a dynamo and a combustion engine, by which the accumulator may be recharged to its previous state after activity (23). The recovery process will be considered more fully in part III of this paper: here we shall deal only with the initial phases of contraction.

It has been shown by Hartree and A. V. Hill (23) that the initial process of contraction must be regarded as occurring in three phases, corresponding to the development, the maintenance and the disappearance (relaxation) of the mechanical response. By an accurate method of analysis of the photographic record of the galvanometer deflection, they proved that, in an isometric contraction, each phase is accompanied by a production of heat, as shown in figure 3. In the case of a single twitch, of course, the second phase (maintenance) is absent. It seems likely that the development of the response is due to the liberation of lactic acid, the lactic acid then proceeding to alter the tension and natural length of the muscle-fiber by some unknown effect of its hydrogen ion. In a twitch relaxation is then caused by the anaerobic chemical removal of the lactic acid from the site of its action. Both processes are accompanied by a production of heat. During prolonged stimulation the "maintenance" phase occurs, and must be accompanied by a continual evolution of heat, owing to the fact that a steady concentration of lactic acid can be maintained at its place of action *only* if the removal-processes be balanced by equal production-processes.

During the development of an isometric contraction heat,  $H_1$ , and potential energy,  $E$ , are produced in the muscle fibers: during relaxation the potential energy,  $E$ , disappears, either as heat, or into some latent molecular form, the lactic acid which excited its appearance being neutralised, or removed, with heat-production  $H_2$ , by some process unknown. The facts, *a*, that this process is accompanied by a production of heat, *b*, that the temperature coefficient of its velocity is very

high, suggest that it is a positive chemical reaction, rather than a physical process such as diffusion away from the active areas (54, p. 310). If, moreover, the lactic acid, once it had done its work, simply collected by physical diffusion in the fluid spaces [Meyerhof's Ermüdungsorten] of the muscle fiber, then there should be a measurable increase of hydrogen ion concentration as the result of even a single twitch. Roaf (64) claimed to have demonstrated such a change by

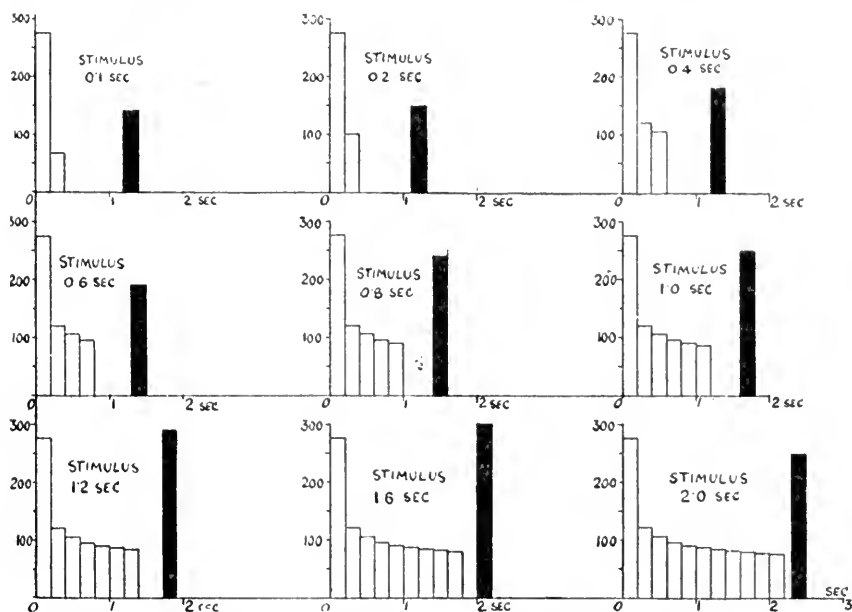


Fig. 3. Heat liberated by sartorius muscles at 0°C. in oxygen. The height of each rectangle represents the heat given out in the interval corresponding to the base on which it stands. Skeleton rectangles represent the heat liberated during contraction, black rectangles the heat liberated during or immediately after relaxation.

the use of an  $\text{MnO}_2$  electrode, but Ritchie (63), repeating Roaf's experiments with great care, found that as the result even of a prolonged tetanus, the change of hydrogen ion concentration inside the muscle is almost negligibly small. Certainly the lactic acid produced in the muscle is neutralised very rapidly, and it is natural therefore to ascribe relaxation to this process of neutralisation.

*The isometric twitch.* The total initial heat-production in a twitch, like the force developed or the maximum work done (see p. 316 above),

is affected by extension of the muscle (16), (12), and by temperature (12), (24). In a maximal isometric twitch, as the initial extension increases the heat increases also, but only to a maximum, after which it diminishes considerably. It might be supposed that the energy-liberation is a surface effect, increasing with the length, or the surface, of the fiber; this, however, fails to explain the existence of the maximum, and the diminution with further extension. A rise of temperature diminishes  $H$ , the heat-production in a twitch, probably for the same reason as it diminishes  $T$ , the force developed (see p. 315 above).

The ratio  $\frac{T}{H}$ , in an isometric twitch, has been studied under a variety of conditions. The effect of temperature upon it seems to be very small; observations by Weizsäcker (67) appeared to show a definite effect, but Hartree and A. V. Hill (25) in carefully controlled experiments were unable to substantiate it. The effect, if any, is very small. Where nearly everything connected with muscular contraction is so largely influenced by temperature, the fact that  $\frac{T}{H}$  is unaffected thereby suggests some special connection between  $T$  and  $H$ . Various observers have shown that there is a proportion between the oxygen-usage of a heart, and the pressure developed in its beats (62). Also, there are various grounds for believing that the potential energy developed is some fraction of  $lT$ ,  $l$  being the length of the muscle, (24), (31), (56). If so, the constancy of  $\frac{T}{H}$  for varying temperature shows that the fraction of the total energy liberated as mechanical potential energy is independent of temperature.

The value of  $\frac{T}{H}$  is affected by the strength of the shock, decreasing as the shock is increased (25, p. 402), and by initial extension, decreasing as the extension is increased (25, p. 400). Following a shock, the value of  $\frac{T}{H}$  for a subsequent shock always passes through a prolonged super-normal phase (25, p. 406), the "efficiency" of a short tetanic contraction being thereby appreciably increased.

As the strength of the shock is increased the heat developed in a twitch, like the maximum force, increases in a series of steps until the maximal shock is reached; it is curious however to find (25, p. 404) that in some cases (but not in all) a *super-maximal shock evokes a smaller*

*heat-production than a maximal one, although the mechanical responses are in size and character identically the same.* Some 15 per cent increase in efficiency therefore can be obtained sometimes by employing super-maximal shocks. The mechanism of this paradoxical result is quite unknown: its possibility, however, together with the facts, *a*, that the time-relations of the isometric twitch are affected by the strength of the shock; and *b*, that  $\frac{T}{H}$  also is so affected, shows that there are dis-

tinct limitations to the "all or none" principle when applied to the twitch of a muscle fiber. If it were true under all circumstances that variation of strength of shock causes simply a variation in the *number* of fibers responding, and not in the character of their response, then none of these phenomena could occur (25, p. 404). Apparently even in a single twitch the response of a muscle fiber can be varied by varying the strength of shock directly applied to it, as well as by varying the extension and the temperature of the muscle; while the phenomena of summation of responses are well known. It seems unwise therefore to regard as a universal principle a rule, which may be generally true of nerve, but which in muscle is so restricted that it appears to have few points of application left.

The thermal response to a shock is not the *result* of a mechanical response, nor indeed is it necessarily accompanied by one. As Weizsäcker (67), (68) showed, the application of a suitable narcotic substance may eliminate the mechanical response completely, while leaving a considerable heat-production. Thus chemical reactions may be released by a shock even though there be no possibility of their evoking the visible changes of a mechanical response. This is good indirect evidence that the production of mechanical energy is due to some physical effect of one of the products, or intermediate products, of the reaction, the mechanism affected by that particular body being liable to be put out of action by the narcotic.

*The prolonged contraction.* The heat produced in a prolonged contraction, occurring in response to a maximal tetanic stimulus, depends upon a variety of factors, the initial extension, temperature and condition of the muscle, the frequency and duration of the stimulus, and the amount of shortening allowed. The last-named has never received adequate investigation, though the means are now available. All the following results refer to isometric contractions. The effects of initial extension are the same as in the simple twitch. The effects of temperature and duration are as shown in figure 4 (24, p. 137), for the unfat-

tigued sartorius of *rana temporaria*. The heat-production increases with the frequency of stimulation, up to the point where complete summation is attained (32), after which it reaches a maximum and remains constant. Fatigue invariably causes a diminished heat-production.

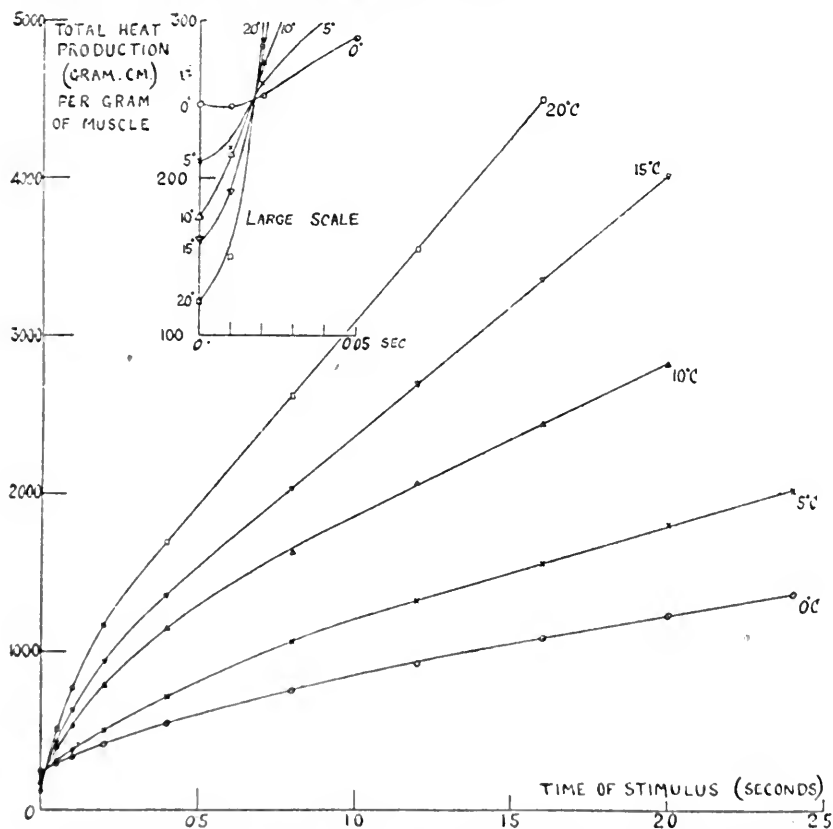


Fig. 4. Relation between heat-production, duration of stimulus and temperature. The large diagram shows the curves up to 2.0 seconds on a small scale; the small diagram shows the initial shape of the curves up to 0.05 second, on ten times the scale. Actual observations shown by dots.

Of these various factors the most important are shown in figure 4. For very short durations the heat is slightly greater at the lower temperature; for long durations it is much greater at the higher temperature; for one particular duration it is the same at all temperatures. (These experiments were made with a frequency of stimulation of 180 per second.) Moreover after a very short while the curves become straight lines; the

heat-production occurs at a steady rate. The steady rate is unaffected by the frequency of stimulation, provided this has more than a certain value; its temperature coefficient however is 2.8 for 10°C., showing that some chemical reaction regulates the rate at which energy is supplied to maintain the contraction (24, p. 139). It seems possible that the immediate precursor of lactic acid, probably a hexose-phosphate, is present in its "ready" form only in very small amount, and has to be reformed from glycogen as required during prolonged stimulation. In that case the speed with which it is reformed would determine the rate of the energy discharge, and we should expect the latter to have a chemical temperature coefficient.

The energetics of prolonged stimulation may be expressed in another way, viz., by plotting  $\frac{H}{T}$  (instead of  $H$ ) against the duration of the stimulus (24, p. 144). If this be done it will be found that the total energy liberated in a prolonged isometric contraction can be split up into two components, one concerned in the *development* of the mechanical potential energy, the other in its *maintenance* at a constant level. Expressed in a mathematical form:

$$H = Tla(1 + bx)$$

where  $H$  is total energy,  $T$  is maximum force developed,  $l$  is length of muscle,  $x$  is duration of stimulus, and  $a$  and  $b$  are constants:  $a$  is independent of temperature,  $b$  is largely affected by it, increasing 2.3 times for 10°C. The quantity  $aTl$  represents the potential energy developed (or some quantity proportional to it); it is noticeable that the value of  $aTl$  agrees reasonably well with the potential energy calculated from the force-extension diagram of the active muscle. The quantity  $bx(aTl)$  represents the energy degraded in a stimulus of duration  $x$ , in *maintaining* a state of potential energy  $aTl$ . The greater  $b$  is, the greater is the wastage of energy in maintaining a constant force. In a fresh, rapidly contracting, or warm muscle,  $b$  is larger; in a fatigued, slowly contracting, or cool muscle,  $b$  is smaller; thus in the latter cases the maintenance of a force is accomplished with the greater economy.

With certain limitations these results apply to human muscles. In the general expression for the total energy liberated:

$$H = aTl(1 + bx)$$

we may write  $aTl = W_0$  (or some quantity proportional to  $W_0$ ), where  $W_0$  is the theoretical maximum work discussed above (p. 311). At a given temperature human muscle appears to contract much less rapidly

than frog's, so that  $b$  is less; at  $37^\circ$  however  $b$  is large enough to make a prolonged contraction fairly uneconomical. It is shown below that in the complete cycle of the contraction, i.e., including the recovery process, there is about  $2\frac{1}{2}$  times as much energy set free as in the initial processes alone. Hence if  $Q$  be the total energy liberated in the complete cycle, we may write:

$$Q = 2.5 W_0 (1 + bx) \dots \dots \dots 1$$

This leads us to a consideration of the mechanical efficiency of human muscles. It is realised that equation I applies literally only to an isometric contraction: it will probably be found however to apply, with a different value of  $b$ , to a muscle undergoing actual shortening. The realisable work  $W$ , has been shown (p. 313) to obey the equation  $W = W_0 \left(1 - \frac{k}{t}\right)$ , where  $k$  is a constant and  $t$  is the time occupied in the shortening. If the contraction be maintained only long enough to cover the actual shortening, i.e., if  $x = t$ , we may write  $W = W_0 \left(1 - \frac{k}{x}\right)$ , so that the mechanical efficiency is

$$\begin{aligned} \frac{W}{Q} &= \frac{W_0 \left(1 - \frac{k}{x}\right)}{2.5 W_0 (1 + bx)} \\ &= \frac{0.4 \left(1 - \frac{k}{x}\right)}{(1 + bx)} \dots \dots \dots \text{II} \end{aligned}$$

If we knew  $k$  and  $b$  we could calculate from equation II the mechanical efficiency of maximal human muscular movements carried out at any speed. A determination of  $k$  can be made as described by A. V. Hill (35). The value of  $b$  might be determined directly by measurements of oxygen-intake: it might also be inferred approximately from thermo-electric observations on small mammalian muscles; at present, however, it can only be arrived at backwards and roughly from a knowledge of the maximum net mechanical efficiency. Repeating the calculation given by A. V. Hill (35) with the rather different symbols and the new value of the recovery heat-production, it can be shown that the maximum mechanical efficiency is obtained for a value of  $x = k \left[1 + \sqrt{1 + \frac{1}{k lb}}\right]$

Putting  $k = 0.24$ , as found by Hill for the biceps and brachialis anticus of a healthy young man, the following table may be calculated:

<i>b</i>	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	1.0
Optimum <i>x</i>	00	1.82	1.37	1.18	1.05	0.97	0.92	0.88	0.84	0.79
Maximum <i>W/Q</i>	0.4	0.295	0.260	0.235	0.220	0.205	0.190	0.180	0.170	0.155

From this table, if we may assume the maximum net efficiency of human muscular movement to be about 0.26, the value of *b* is about 0.2. Taking this value for the sake of calculation (though the actual value makes no difference to the following argument) the efficiency of a maximal human muscular movement becomes

$$\frac{W}{Q} = \frac{0.4 \left(1 - \frac{0.24}{x}\right)}{(1 + 0.2x)}$$

This relation between the efficiency and the time occupied in the movement, is shown in figure 5. It is seen that  $\frac{W}{Q}$  rises rapidly from zero at time 0.24 second (the duration of an unloaded contraction), attains a maximum of 0.26 at 1.37 second, and then slowly falls again to zero. Thus human muscles have an optimum speed of working: a higher speed seriously interferes with their efficiency, a lower speed interferes indeed, but much less. It should not of course be supposed that the numbers given here are all absolute constants; they depend upon the characteristics of the actual muscles used; there can, however, be little doubt that the efficiency of human muscular movement is governed, in general, by considerations of the kind advanced above. It can be shown moreover (35) that a submaximal effort is less efficient than a similar maximal one, occurring in the same time against a heavier load. This is of importance as show the inadmissibility, in ergometer experiments, of adopting as "base-line" the energy expenditure at a lower level of work. (For example, see Benedict and Cathcart (3).) The whole subject would seem to be of considerable importance from the point of view of the optimum conditions for muscular efficiency in the processes of industry or athletics, and especially in relation to physiological experiments with ergometers. We cannot, however, discuss it further here.

An excellent short account of the *Thermodynamics of Muscles* has been given by Meyerhof (51).

PART III. THE RECOVERY PROCESS. *Isolated muscles.* The discoveries *a*, of Liebig (42) that an atmosphere of oxygen preserves the irritability of an excised resting muscle, and *b*, of Ludwig and Schmidt (45) that

oxygen delays the onset of fatigue in a similar excited muscle, were followed by the proof by Fletcher (18) that a muscle may be preserved indefinitely from the onset of rigor mortis, or indeed recalled from its incipient stages, by immersion in oxygen. These researches, culminating in the classical investigation by Fletcher and Hopkins (20) of lactic acid in muscle, led the present writer (30) in 1913 to determine

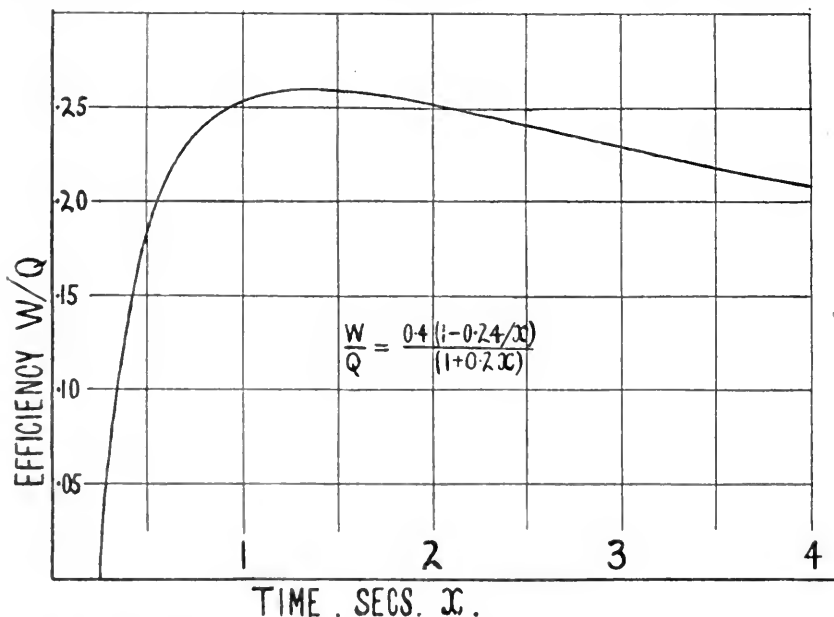


Fig. 5. Mechanical efficiency of human muscular movement in relation to the duration,  $x$ , of the contraction.

the heat-production during the recovery phase. It was found, in the presence of oxygen, that the production of heat is prolonged for some minutes after excitation, the total "delayed" heat being of the same order of magnitude as the "initial" heat; while, in the absence of oxygen, the delayed heat-production was much smaller, or (as was supposed then) negligible. This, together with the proof that the "initial heat" has a non-oxidative origin, makes it clear that the muscle is similar to an accumulator, that it contains potential energy ready for discharge, which is restored by some oxidative process during recovery. What is the nature of this recovery process, and is it the oxidation of lactic acid?

Experiments by A. V. Hill (29) and by Peters (61) had proved that the production of 1 gram of lactic acid in muscle, whether in rigor or as the result of exercise in the absence of oxygen, is accompanied by the liberation of about 450 calories. Later and more exact experiments by Meyerhof (53) have shown that this value is rather too high, that in rigor, or in fatigue induced by a succession of single shocks or short tetani, the value should be about 350 calories<sup>1</sup> per gram of lactic acid; the difference does not affect the argument. If, as A. V. Hill (30) estimated, the oxidative recovery process involved about as much heat-production as the initial process, the total heat set free in the complete cycle of liberating and removing 1 gram of lactic acid had to be about  $2 \times 450 = 900$  calories; later and much more accurate observations by Hartree and A. V. Hill (26) have shown that the average value of the total recovery heat is about 1.5 times the initial heat; accepting this figure, together with Meyerhof's more accurate determination, the total energy liberated in the complete cycle is  $2.5 \times 350 = 875$  calories. This is close to the 900 calories originally estimated. Thus in the liberation and the subsequent oxidative removal of 1 gram of lactic acid 875 calories have been produced, and the muscle is finally in the same state as before, except that some of its glycogen has been oxidised. The heat of combustion of 1 gram of lactic acid is 3661 calories, while that of the corresponding amount (0.9 gram) of glycogen is 3772 calories;<sup>1</sup> the total heat liberated in the production and the subsequent oxidative removal of 1 gram of lactic acid is only 24 per cent of the former and 23 per cent of the latter. It was obvious therefore that, unless there were some serious error, the whole of the lactic acid was not removed by the simple process of oxidation, that for every 1 gram of lactic acid oxidised in the recovery phase, some 3 more grams of lactic acid were removed by some other non-oxidative mechanism. There seemed no escape from this conclusion. In 1915, however, Parnas (58) published experiments purporting to show that during the oxidative recovery phase a complete equivalence existed between the oxygen used and the lactic acid removed. These experiments were accepted by Fletcher and Hopkins (21) in their Croonian Lecture, and at first by Meyerhof (52), and it was assumed in consequence that in the recovery phase the whole of the lactic acid was really oxidised, and not, as had been supposed, restored to its previous position ready for subsequent activity. There remained, however, the

<sup>1</sup> Since this article was completed the writer has received the manuscript of a forthcoming article by Meyerhof, in which this figure is modified slightly. See Appendix.

fundamental difficulty that on this view the recovery heat-production was quite inadequate. From this impasse the situation has been relieved by the brilliant investigations of Meyerhof. Meyerhof first (54) repeated the experiments of Parnas with entirely the contrary result. Allowing for the oxygen used in the same period by similar resting muscles he found that *during the recovery period of stimulated muscles an excess of oxygen is used which is insufficient to account for the oxidation of more than  $\frac{1}{3}$  to  $\frac{1}{4}$  of the lactic acid which can be shown, by direct determination, to have disappeared.* This is in excellent agreement with the results of the calculation from the recovery heat-production. Meyerhof repeated his measurements of the oxygen used in the recovery process under various conditions, always with the same result, viz., that not more than  $\frac{1}{3}$  to  $\frac{1}{4}$  of the lactic acid is removed by direct oxidation. It is difficult not to attribute Parnas' results to some unknown experimental error. Meyerhof moreover has confirmed his results by a variety of other observations. In the first place he found (52) that in the recovery removal of lactic acid the volume of  $\text{CO}_2$  produced is almost exactly equal to that of  $\text{O}_2$  absorbed; the respiratory quotient is unity. This points to the oxidation either of lactic acid or of carbohydrate. Further, by direct measurement (in a calorimeter) of the recovery heat-production after prolonged stimulation, he found (54) that the heat is less than that calculated from the oxygen used by an amount equivalent to the energy liberated in the initial stages of the contraction; in other words, the energy liberated by oxidation in the recovery process partly appears as heat, and partly is absorbed in some physico-chemical process restoring the muscle to its original condition. Thus the heat set free plus the energy re-absorbed is shown, by direct measurement, to correspond to the oxygen used; and this oxygen corresponds to the oxidation of an amount of lactic acid only  $\frac{1}{3}$  to  $\frac{1}{4}$  of that known actually to have been removed. Finally Meyerhof has shown (54), (55), again in contradiction of Parnas (58), that (making due allowance for the oxidation of a quantity corresponding to the measured amount of oxygen used in survival or recovery) whenever lactic acid appears a corresponding amount of glycogen disappears, and that whenever lactic acid disappears a corresponding amount of glycogen can be recovered. It seems therefore quite certain, *a*, that the recovery process consists of an oxidation (either of lactic acid or of carbohydrate), of which part of the energy appears as heat, part is absorbed in restoring the muscle to its original condition of readiness for mechanical activity; *b*, that in the initial process of contraction glycogen, or some product of glycogen, is changed

explosively into lactic acid; *c*, that in the recovery process the glycogen (or its product) is restored and the lactic acid removed; and *d*, that the oxygen used in recovery is employed in oxidising carbohydrate (or lactic acid) in amount equivalent to about  $\frac{1}{4}$  of the lactic acid removed.

Experiments have recently been made by Hartree and A. V. Hill (26), with particular care to ensure accuracy, to determine both the extent and the time-relations of the recovery heat-production. The

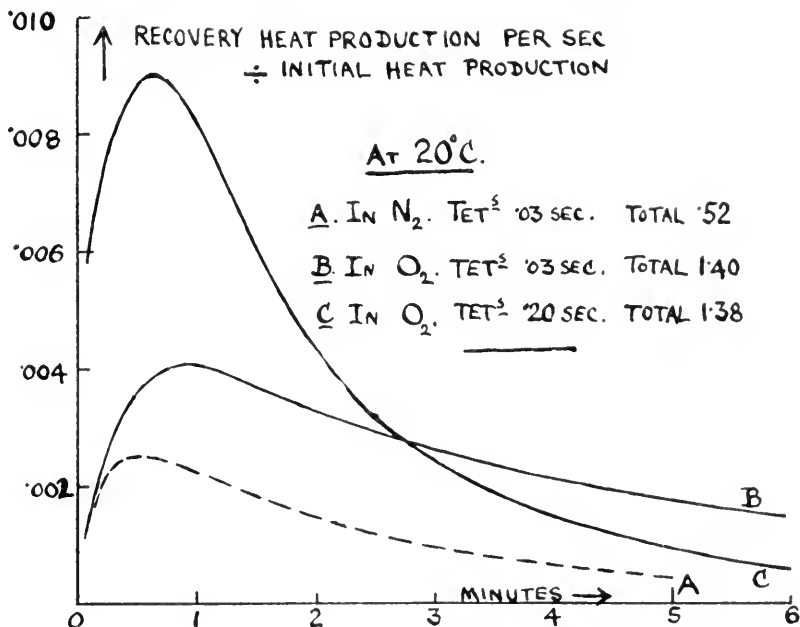


Fig. 6. Rate of production of heat during recovery of sartorius of *rana temp.* in  $O_2$  and in  $N_2$ . Note that the heat is expressed, not in absolute units, but as a fraction of the initial heat-production.

rate of recovery heat-production appears to follow a perfectly definite time-course, as shown in figure 6; it starts at a low level immediately following the contraction, rises to a maximum, and then slowly falls to zero, the last part of its curve being of an exponential character. It is noticeable that the longer stimulus, evoking the more extensive liberation of energy, gives a recovery heat-production which is not only absolutely but relatively more rapid than that given by the shorter stimulus. When the velocity of a chemical reaction increases more rapidly than the first power of the concentration of one of its reacting

bodies we conclude that the "order" of the reaction is greater than unity, that we are dealing with a bimolecular or a termolecular reaction. All the experiments made confirm the conclusion that the "order" of the reaction occurring in the recovery process is greater than unity, a conclusion which is entirely in keeping with the view advanced by Meyerhof (54), on the basis of Embden's work, that in the recovery process two molecules of lactic acid are united to make one molecule of glucose, which is then combined with phosphoric acid to form a hexose diphosphate. The rate of the recovery heat-production is also affected by temperature, increasing rapidly as the temperature rises. The cause of the maximum on the curve, until we have further knowledge of the chemical reactions occurring in recovery, must remain a matter of conjecture; were these chemical reactions of a simple kind we should expect an initial high rate followed by a continuous fall to zero; clearly we are dealing here with complex reactions whose full activity is not manifest until some time after their commencement. Possibly the time-relations of the heat-production during recovery may give some clue as to the actual chemical mechanism of the latter.

In the experiments of Hartree and A. V. Hill (26) the special precautions taken to ensure a steady zero made it possible to measure the recovery heat-production after a short tetanus (0.3 to 0.5 second), for some 6 or more minutes, and so to arrive at an accurate estimate of its total value. Calling the total initial heat-production  $H_0$  and the total recovery heat-production  $H_r$ , the ratio  $\frac{H_r}{H_0}$ , in frog's muscles in oxygen at 20°C., had values varying from 1.0 to 2.0, (the majority lying between 1.4 and 1.6) and a mean of 1.5. The ratio was independent of the duration of the stimulus. In the absence of oxygen, i.e., after some hours in  $O_2$ -free nitrogen, with preliminary stimuli to use up any traces of oxygen still present, the delayed heat-production was not abolished altogether but was still perceptible as shown in figure 6. The total value of the delayed heat in this case bore to the initial heat a ratio varying from 0.3 to 0.6, with a mean of about 0.4. This might perhaps be attributed to oxygen still present dissolved in the muscle, although all precautions had been taken to avoid this possibility; but a thin sartorius muscle soaked in a KCN solution (0.0007 n to 0.002 n) for 1 minute or more, in which presumably all oxidations had been eliminated, still showed a delayed heat-production amounting to some 30 per cent of the initial heat. If further investigations confirm the existence of this smaller anaerobic delayed heat-production we shall have to assume either

1, that the chemical reactions of the recovery process can progress to some degree in the absence of oxygen, and get held up only at the oxidative stage; or 2, that in the muscle there exists some hydrogen acceptor capable, like the dipeptide isolated by Hopkins (36), of allowing oxidations to proceed in part even in the absence of molecular oxygen; or 3, that the heat liberated in the absence of oxygen should be regarded as belonging to the initial stage (possibly relaxation), and not to the recovery stage at all. There seems at present to be insufficient evidence to allow us to distinguish between these three alternatives.<sup>2</sup>

The existence of this anaerobic delayed heat-production may upset some of the calculations given above. In anaerobic activity, or in rigor, the heat measured by Meyerhof, for comparison with the lactic acid formed, is the same as the sum of the initial and the delayed heats measured by Hartree and Hill in the absence of oxygen. Taking the "initial heat" as 100, the delayed heat in the absence of oxygen is 40, while the recovery heat in the presence of oxygen is an extra 110. In this case the total energy<sup>2</sup> of the oxidative phase is not 2.5 times, but only 1.8 times the total heat of the combined anaerobic phases. This will only strengthen the argument in favor of the non-oxidative removal of lactic acid, but without further evidence as to the mechanism of this post-activity non-oxidative heat-production it will be useless further to discuss the matter here.<sup>3</sup>

*Unisolated muscle.* Verzàr (66), observing the oxygen-usage of the gastrocnemius of an anesthetised cat, found that during stimulation the rate of oxygen-intake is not increased, but that after stimulation it rises and remains for a long time above its resting level. The reason for the absence of a rise during stimulation is presumably, as Lindhard (43) found for man during "static work," that the blood supply is hindered by the hardening of the active muscle. Barcroft and Kato (2) performed similar experiments on the gastrocnemius of anesthetised dogs, employing rhythmic instead of continuous stimulation. They found a rise of oxygen-intake during stimulation (the blood supply not being stopped by rhythmic excitation) but a still greater rise lasting for hours after the stimulus was over. These experiments contrast curiously with those on man (see below), in that the recovery process is so prolonged; after several hours the return to resting level is not as complete as it is, in man, after as many minutes. Whether this be due to excessive stimulation, or to the effect of the anesthetic, one cannot say. There is

<sup>2</sup> That is, heat plus restored potential energy.

<sup>3</sup> See Appendix.

no doubt, however, that such experiments do not represent the normal processes of recovery in warm-blooded animals, which are better studied in man.

*Man.* Owing to the lag in a calorimetric method, and to the errors inherent in calculation from the  $\text{CO}_2$ -output during a rapidly changing metabolism, the only practicable means of following the recovery process in man is that of determining the oxygen-intake. In their experiments on Pike's Peak (13, p. 258) Douglas, Haldane, Henderson and Schneider made preliminary observations, by the Douglas bag method, of the course and extent of the oxygen-intake during recovery from exercise in man. These experiments have been confirmed and extended by Campbell, Douglas and Hobson (7), and recently by Lupton (46), also using the Douglas bag method, the former investigating the recovery period after work on a bicycle ergometer, the latter after various other forms of exercise. Krogh and Lindhard also (40) have investigated the oxygen-intake after ergometer exercise, and Lindhard (43), (44) after the "static work" of supporting the body in various positions, customary in a gymnasium. In healthy men recovery is very rapid, the primary return to a resting level of oxygen-intake being complete within 10 minutes of the end of exercise, and almost complete within 3 or 4 minutes. Even after an exhausting effort recovery is not sensibly prolonged, though there may be a small increase in the resting level of oxygen-intake, corresponding possibly to a rise of body temperature, possibly to a restoration of glycogen in the muscle.

At  $20^\circ\text{C}$ . the recovery heat-production of a frog's sartorius in oxygen is nearly complete in 6 minutes (see fig. 6), 50 per cent complete within 2 minutes. Hartree and A. V. Hill (26) found the recovery heat-production to be considerably affected by temperature, though they could not determine a temperature coefficient. Assuming the latter to be about 2.4, the recovery process at  $37^\circ\text{C}$ . should be nearly complete within  $1\frac{1}{2}$  minutes, and 50 per cent complete within 30 seconds. In man the oxygen pressure is not so high, and we might expect the rate of recovery to be less. Hence the speed of oxidative recovery is of the same order of size in man as in isolated muscle. After prolonged stimulation of large masses of isolated muscle the speed of recovery is determined by the rate of oxygen diffusion, and is therefore very slow; where, however, the oxygen supply is adequate, and especially at a high temperature, oxidative recovery is a fairly rapid process.

Muscular exercise may be long continued, or short, while the chemical processes of recovery may occur mainly during the exercise, or wholly

after it. At the beginning of exertion an oxygen deficit is built up which is compensated only at the end. In prolonged exertion the greater part of the recovery process is completed during the exercise itself; during uniform exertion a steady state is rapidly reached in which the total oxygen deficit becomes constant. In this case the total oxygen absorbed in the post-exercise recovery process is equal to that used in not more than  $\frac{1}{2}$  to 1 minute of the previous exertion. This shows the extreme rapidity of the oxidative recovery process in normal man. The highest possible rate of oxygen-intake in man, during steady exercise, is about 6000 cc. per minute; a total therefore of something like 4000 cc. may be used in recovery. Assuming that 22.2 liters of oxygen oxidise 30 grams of lactic acid or glucose, and correspond therefore (from above) to the removal, during recovery, of about 120 grams of lactic acid, we see that during and immediately after severe exercise, there must be something of the order of 22 grams of lactic acid present in the body. If the active muscles be assumed, for the sake of calculation, to weigh 16 kilos, this corresponds to a lactic acid concentration of about 0.13 per cent, an amount which is about  $\frac{1}{2}$  of that found in isolated frog's muscles after severe anaerobic stimulation. These 22 grams of lactic acid we may suppose neutralised by bicarbonate present in the blood or muscles; in the process it must turn out some 16 liters of  $\text{CO}_2$ , while during its oxidative removal in recovery the same amount of  $\text{CO}_2$  must finally be retained by the body. It is small wonder, therefore, either that lactic acid may appear in the blood and urine, or that the combined  $\text{CO}_2$  of the blood may be altered (8), or that the respiratory quotient may exhibit violent fluctuations, as the results of severe exercise and during recovery therefrom (7, p. 36); (46). These fluctuations must render any attempt to follow the rapidly changing metabolism in such cases, by means of measurements of the  $\text{CO}_2$  output, entirely useless.

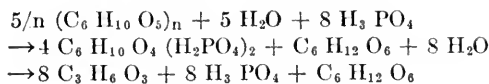
Lupton (46) has investigated also the recovery from short bouts of very violent exercise, of a nature too severe to be prolonged. In the extreme case of 10 seconds of such exercise, during which the subject holds his breath, practically the whole of the oxygen used after exercise in excess of the resting level, must be due to recovery, and Lupton found values as high as 2000 cc., as the total oxygen usage of recovery from 10 seconds exercise. This corresponds to a production of about 11 grams of lactic acid, a concentration roughly of the order of 0.05 per cent averaged over all the muscles of the body. This amount of oxygen used in the oxidation of carbohydrate yields about 10,000 calories, enough to raise the body-temperature of an 80 kilo man

some  $0.15^{\circ}\text{C}$ . Thus the most extreme form of exercise causes, per second of the exercise, a total liberation of energy of 1000 calories. This, if continued, would be equivalent to an expenditure of 4.18 kilowatts or of  $5\frac{1}{2}$  horse-power, a rate which it would clearly be impossible for a man to maintain for more than 20 or 30 seconds. The total oxygen dissolved in the fluids of the body of an 80 kilo man, at the resting venous oxygen pressure, say at 50 mm. Hg, is about 100 cc.; the total oxygen combined with hemoglobin in his blood is not more than 800 cc.; while the rate of oxygen supply by the circulation, even after it has been pushed to its extreme limit by continued exertion, cannot much exceed 6000 cc. per minute. Consequently the most violent exercise of which a man is capable may in 5 seconds lead to breakdowns requiring recovery oxidations sufficient to exhaust all the molecular oxygen available in the body; and since such exertion may be continued certainly for 20 seconds the body may, so to speak, go into debt for oxygen to the extent of 3000 or 4000 cc. It is this fact alone which makes it possible for the body to carry out the more violent forms of exertion; were it necessary for the oxygen supply to keep pace with the exertion the capacity of the body for short-lived violent effort would be reduced to one-half or less. The same fundamental importance of recovery oxidations is shown by the experiments of Lindhard (43), (44). Here the subject, for example, maintained his body-weight for about 1 minute with arms bent, the rate of oxygen-intake being measured during, and at various moments after the exercise. In one experiment the total increased oxygen-intake, as the result of the exercise, was 1125 cc., of which only 168 cc. were used during the working period itself. Lindhard explains the lowness of the oxygen-intake during the working period as due mainly to the rigidity of the active muscles placing a severe restraint upon the circulation in them, and so restricting their oxygen supply. The fact that nearly 1000 cc. of oxygen are required in the recovery of what is really a very limited mass of muscles, sufficiently explains the extreme fatigue occasioned by this type of "static work;" this quantity of oxygen is required to remove about  $5\frac{1}{2}$  grams of lactic acid; the muscles involved probably do not weigh more than 2 kilos; consequently a concentration is attained of some 0.2 per cent to 0.3 per cent—somewhere near the maximum amount attainable by stimulation.

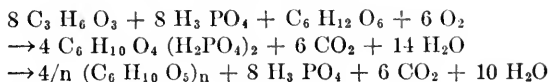
PART IV. THE PLACE OF LACTIC ACID IN THE MECHANISM: The *lactic acid maximum* established by Fletcher and Hopkins (20), the fact that the heat-production associated with the anaerobic formation of lactic acid shows the same phenomena (29), and that this heat-production is

3 to 4 times<sup>1</sup> as great as that obtainable from the breakdown of glycogen into lactic acid, led various writers (and especially the present one) to assume the existence in muscle, in limited amount, of some lactic acid "precursor" possessing stores of energy available for release in muscular activity, this precursor being restored during oxidative recovery (33). Recently however evidence has accumulated that the lactic acid maximum is due to a process of "self-inhibition," the formation of acid being stopped by the rising hydrogen ion concentration (41), (53). If the rise of hydrogen ion concentration be prevented in any way, e.g., by alkali, or by diffusion of the lactic acid into surrounding Ringer's solution, the formation of acid may reach a considerably higher level. The same thing is borne out by the experiments of Meyerhof (56), who found the total mechanical response (i.e., to complete fatigue), as also the acid maximum, to be increased in an alkaline medium. Hence one aspect of the case for such a type of lactic acid precursor completely disappears. Moreover the evidence has become overwhelmingly strong<sup>4</sup> that lactic acid arises, possibly not directly, from glycogen (59), (55), and the work of the Embden School (14), (15), which there is no space to discuss here, seems to show that some hexose-diphosphate is a link in the chain by which glycogen and lactic acid are mutually transformable into one another. According to Meyerhof (55), the reaction may be formulated as follows (writing glycogen as  $(C_6H_{10}O_5)_n$ ).

*Anaerobic breakdown*



*Oxidative recovery*



If this be so we are left with one fundamental problem, the fact that the heat-production in the anaerobic phase is much greater, and in the oxidative recovery phase much less, than corresponds to the chemical reactions written down. Landolt and Börnstein give the heat of combustion of glycogen as 4191 calories per gram, and of lactic acid as 3661 calories.<sup>1</sup> Thus 0.9 gram of glycogen forms 1 gram of lactic acid with

<sup>4</sup>Since the above was written a paper has appeared by Foster and Moyle, *Biochem. Journ.*, 1921, xv, 672, which strongly confirms this conclusion. See also Hopkins' Herter Lecture, No. 2, *Johns Hopkins Hosp. Bull.*, 1921, xxxii, 359.

the evolution of only 109 calories, an amount only  $\frac{1}{3}$  to  $\frac{1}{4}$  of that actually appearing in the anaerobic formation of 1 gram of lactic acid in muscle; there is an excess of some 250 calories to account for. In the recovery phase there is a similar deficit. It is natural to attribute this alternating excess and deficit to the forward and backward progress of some reaction directly connected with the mechanical response. In the complete anaerobic cycle of an isometric contraction and relaxation no external work has been done and nothing has happened, so far as we know, except that a certain amount of lactic acid has appeared. Any action of the lactic acid, or its hydrogen ion, on sensitive surfaces (*Verkürzungsorten* (Meyerhof)) during contraction has been reversed during relaxation, a conclusion which is borne out by the fact that relaxation is accompanied by heat-production (23). Everything is as it was, except that some glycogen has disappeared and some lactic acid has appeared, not indeed free acid, as Ritchie (63) has shown, but acid in some combined or neutralised form. There seems no escape from the conclusion that the combination, or neutralisation, of the acid is responsible for the extra 250 calories appearing in the anaerobic phase, and that the reversal of this combination, or neutralisation, is responsible for the similar deficit in the oxidative recovery phase (53, p. 273).

The neutralisation of lactic acid with bicarbonate involves only a very small quantity of heat, according to an estimation by A. V. Hill (33, p. 371) only about 27 calories per gram of lactic acid. This is not sufficient to account for the excess and deficit under consideration. The neutralisation of the acid with free base (KOH) would supply us with enough heat, but there is no free base present. It may be, however, that in the fluids of the muscle there is some alkaline protein body capable of combining with and neutralising the lactic acid with the required evolution of heat.<sup>2</sup> In any case the process by which the lactic acid is fixed must be one possessing considerable free energy, since it has to reverse the very vigorous one in which lactic acid reacts with the sensitive surfaces of the muscle, producing free energy in the mechanical form.

In this way the vain search for a lactic acid "precursor," possessing stores of potential energy ready for subsequent release, has been replaced by an attempt to find the chemical mechanism of relaxation, the means by which lactic acid, having effected the development of tension, is removed from the site of its action, so allowing the previous state to reappear.

In a recent summary (38) J. v. Kries has sketched the following picture of the manner in which muscular contraction occurs: *a*, *contraction* is due to physico-chemical forces employing a form of energy present in amount only sufficient for one maximal contraction; *b*, in *relaxation* this form of energy is restored by forces which may be described as the affinity of some intermediate body for lactic acid; this body is present in amount sufficient to produce relaxation for a number of contractions; and *c*, in *recovery*, the energy of the intermediate body is restored by oxidative processes. It will be seen that this sketch follows very closely the lines suggested above.

An excellent short account of the rôle of lactic acid in muscular contraction has been given by Meyerhof (50).

#### APPENDIX

Since the above pages were completed the writer has been able to see the manuscript of a paper by Meyerhof (*Energieumwandlungen VI*) shortly to appear in Pflüger's Archives. The conclusions in this paper are so pertinent to the discussion here that they have been included in this appendix. First, Meyerhof has redetermined the heat of combustion of lactic acid, and finds it appreciably different from that given in books of chemical tables. He finds that the formation and neutralization (with phosphate or bicarbonate) of 1 gram of lactic acid from glycogen leads to an evolution of about 190 calories, a considerable increase on that estimated above. He finds also that in intact muscle the same amount of lactic acid is formed anaerobically from glycogen with an evolution of about 370 calories. If the acid be allowed to escape into a surrounding alkaline fluid, this value is considerably reduced, showing that something more than neutralization with an alkaline salt occurs in the living muscle. If lactic acid be formed from glycogen in crushed muscle the heat corresponds closely to the 190 calories, showing that here we have merely the formation and simple neutralization of lactic acid. If moreover an acid be allowed to penetrate into a muscle, without the liberation of lactic acid, the heat produced is about half of that accompanying an equal production of lactic acid in the live muscle. It is clear therefore that the presence of the lactic acid in the muscle leads to an evolution of about 180 calories beyond that caused in its production from glycogen. Meyerhof supposes that this arises from the heat of dissociation of the muscle proteins, and he has shown that, although the neutralization of 1 gram of lactic acid by phosphate or bicarbonate yields only about 20 calories, the addition of the same acid to buffered amino acid solutions yields some 6 or 7 times as much heat.

It is very probable therefore that the remaining  $370 - 190 = 180$  calories must be attributed to some reaction between the acid and the protein constituents of the sarcoplasm. If this reaction be a slow one it may account for the delayed anaerobic heat shown in figure 6: and in this case the process of relaxation may be ascribed to the immediate neutralization of the acid by alkaline salts, the acid then being slowly removed by the proteins, thereby leaving the alkaline salts free to function in later relaxations.

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## THE RELATION OF THE ADRENALS TO THE CIRCULATION<sup>1</sup>

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The relation of the adrenal glands to the circulation presents four major questions. How is the circulation affected by adrenal deficiency and by the administration of adrenal extracts? Do the adrenal glands at any time produce sufficient adrenin to affect the circulation? If so, what factors condition adrenin discharge?

That extensive destruction of suprarenal tissue leads to marked circulatory asthenia has been recognized since the work of Addison and of Brown-Séquard. Profound asthenia leading to death invariably follows complete destruction of all the adrenal tissue. The causation of the cardio-vascular collapse has been studied by many investigators but is still undetermined. Until the epoch making work of Oliver and Schafer in 1896 it was believed that adrenal destruction results in a fatal accumulation of toxic products in the blood; without ever having been conclusively disproved, this theory has largely dropped from view because of lack of satisfactory evidence in its support.

When, however, the remarkable power of adrenal extract as a cardio-vascular stimulant was discovered, it was immediately assumed and for years generally believed that the essential factor in the symptomatology of adrenal deficiency is deprivation of the so-called active principle, now widely known as adrenalin. It was assumed that this substance is constantly discharged in sufficient quantity to maintain a stimulating influence on the sympathetic nervous system. Blood

<sup>1</sup>The literature now<sup>1</sup> includes hundreds of papers dealing directly or indirectly with the relation of the adrenal glands to the circulation. Spacial limitations obviously preclude specific consideration of most of these. More detailed consideration of much of this literature may be found in the new editions of Biedl's and of Vincent's well known monographs and in *Internal Secretions and Metabolism*, edited by Barker, Hoskins and Mosenthal, all of which will supposedly be available by the time this article is in print.

pressure, which changes quickly under the influence of adrenin, has been used largely as a criterion of sympathetic stimulation.

EFFECTS OF ADRENAL DEFICIENCY. The precise circulatory effect of adrenal deficiency was first studied by Strehl and Weiss (1). Their method was to register changes of arterial pressure ensuing upon occlusion of the lumbo-adrenal vein in the rabbit. They noted most commonly a brief fall, which returned to normal upon releasing the vein. Their results were not consistent and are not convincing in that the period of occlusion was very brief and that the depressor effects of sensory stimulation in the adrenal region were not recognized. Young and Lehman (2), in Vincent's laboratory, repeated the experiments on dogs, leaving the ligatures in place from 10 to 30 minutes. But very slight fall of blood pressure occurred and that very gradually. Young (3) independently repeated the experiment and observed no significant fall of pressure for hours. Biedl (4) reported that in case of the adrenals transposed to an extraperitoneal situs, extirpation led to a fall of blood pressure that lasted 15 to 30 minutes, but the pressure then returned to normal and remained so for 2 or 3 days.

In 1912 Hoskins and McClure (5) repeated the ligation experiments on dogs. Occluding ligatures were placed across the mouths of the lumbo-adrenal veins and, after waiting for the immediate effects of the operation to disappear, the results of closing and releasing the veins were recorded. Each experiment continued from 10 to 30 minutes. In only one case was any significant fall of blood pressure observed. Vincent and his students (6) have recently restudied the problem by comparing, over a period of from 12 to 40 hours, the course of blood pressure of anesthetized dogs with adrenals intact and those with the glands extirpated. No significant differences in blood pressure changes were noted after adrenal ligation. Bazett (7) has also found in case of cats and rabbits that adrenal extirpation fails to produce prompt fall of blood pressure. Since injected adrenin has but an evanescent effect, such observations indicate that the symptoms of adrenal deficiency are not due to lack of adrenin. That adrenal extirpation, however, results in no early significant alteration cannot, in the light of recent observations, be maintained. Winkin (8) has shown that the blood pressure reactions to cerebral anemia are modified early after occlusion of the adrenal vessels.

As to the cause of collapse following adrenal extirpation, Elliott (9) proposed a theory that minute amounts of circulating adrenin are necessary to the functioning of the sympathetic system; that is, that,

without actually stimulating, they render it capable of transmitting impulses. This possibility was put to experimental test by Hoskins and Rowley (10). In a series of dogs, either normal or deprived of their adrenals, the effects of slow infusions of dilute "adrenalin" were studied. At no time was satisfactory evidence obtained that augmented adrenin in any way facilitated the transmission of vasomotor impulses. The condition of the vasomotor mechanism was studied by peripheral faradization of sciatic and splanchnic nerves and by injections of nicotin and "adrenalin." In numerous cases the adrenin infusion lessened the vasomotor irritability—sometimes to a marked degree. This was true of both the pressor and depressor mechanisms. The asthenia of adrenal deprivation does not depend, therefore, upon the failure of the sympathetic as a conducting mechanism. Neither does it depend upon a loss of sympathetic irritability, either central or peripheral, as was shown (11) by a study on a series of animals deprived of their adrenals. At a time when the animals exhibited marked weakness, cardiac and skeletal, reflex hypertension from central stimulation of the crural nerve persisted as did pressor reactions to adrenin. The reactions to nicotin, which is a selective sympathetic stimulant, were often even exaggerated. The irritability of the inhibitory fibers to the gut persisted even longer than that of the pressor fibers. That some part of the circulatory mechanism is early impaired, however, is indicated by the recent work of Rich (12), who found that hypotension follows double epinephrectomy within a period of from 4 to 7 hours, at a time when muscular strength is still retained.

That adrenin loss, itself, ordinarily plays any significant rôle in adrenal deficiency is further rendered doubtful by the fact that extirpation of one gland and denervation of the other has been shown by Stewart and Rogoff (13) to result in no apparent difference in the health of the animal, even though the adrenin output was reduced below detectable limits in the efferent adrenal vessels, hence, in the general blood stream, far below the quantity necessary to any known pharmacodynamic reaction. That adrenal denervation leaves an animal handicapped in meeting the strain of severe muscular exertion, however, has recently been shown by Hartman, Waite and Powell (14).

On the whole, it is doubtful whether the striking hypotensive effects of adrenal deficiency have their origin within the sympathetic system. Recent work of Sandiford (15) and others showing the marked effect of adrenin on basal metabolism suggests that the fault, if connected in any way with adrenin deficiency, lies in the effector rather than the conductor mechanism.

THE EFFECTS OF ADRENIN ADMINISTRATION. The outstanding results of intravenous injections of adrenal extract were accurately observed by Oliver and Schafer. In short, they noted that blood pressure is strikingly augmented, the heart stimulated and the blood driven from the splanchnic organs. As to what became of the blood thus shifted from the viscera, their original reports gave little evidence. This mass shifting seems now, however, to be the most significant feature of adrenin hemodynamics. Hartman (16) was the first to study carefully this problem, as such. His general plan of procedure was to administer standard doses of adrenin to animals in three conditions: *a*, with circulation intact; *b*, with the major splanchnic vessels ligated; and *c*, with the major extrasplanchnic vessels ligated. It was found in general that when the blood traversed the extrasplanchnic circuit, depressor effects, hence vasodilatation, resulted from the administration of adrenin; whereas when the circulation was confined predominately to the splanchnic area, the standard dose caused a rise of pressure, hence vasoconstriction.

Numerous other studies of the detailed effects of adrenin in the splanchnic area have been made by plethysmography, venous outflow and Stromuhr methods. The literature on this point has recently been summarized elsewhere (17). Suffice it to say that later work in general has confirmed the observations of Oliver and Schafer to the effect that pressor doses of adrenin result in depletion of the splanchnic domain.

Hartman's studies left undetermined the problem as to what particular structures receive the blood deflected from the splanchnic area. Since the volume of the central nervous system and of the bones is essentially fixed, and since the glandular structures in the extrasplanchnic areas are of relatively slight volume, the mass shifting of the blood must involve predominately the muscle, the skin, or both. Earlier observers had noted that ordinarily the limbs react by contraction; now and again, however, expansion, which was regarded as a passive effect, was seen.

The differential effects of adrenin in the skin and muscle were studied in the writer's laboratory (18). In dogs, studies on the volume of intact limbs and on carotid blood pressure were made simultaneously. Both pressor and depressor doses of adrenin were administered by vein. In most cases the volume of the limb decreased, irrespective of the systemic reaction. In such preparations skin and bone constitute a large proportion of the tissue mass. The effect of disarticulating the limb at the tibio-tarsal joint and removing the paw and the skin

from the plethysmograph was striking. In all cases except when massive doses of adrenin were injected clean-cut expansion now resulted. The studies were extended by noting simultaneously the rate of outflow from a muscular and a cutaneous branch of the same vein. Within all ranges of dosage that could be regarded as physiological, whether pressor or depressor, increased outflow from the muscular branches and decreased outflow from the cutaneous branches were seen. These observations were subsequently confirmed by Hartman and Fraser (19) and by Gruber (20). Gruber further found that cutting the nerve to the muscle prevented vasodilatation during the earlier period in which local vasomotor tonus was abolished, but as tonus was regained dilatation could again be evoked. The outstanding effect of adrenin within physiologic limits, then, is to shift the blood from the splanchnic area and the skin to the skeletal muscles. This effect is seen irrespective of whether pressor or depressor doses are used, hence cannot be regarded as passive.

*Vasodilator effects of adrenin.* That adrenal extract may produce a fall in blood pressure was reported by Moore and Purinton (21) in 1900. Aqueous extracts were made from both the cortex and the medulla of the glands. The protein components were largely removed by boiling in a slightly acid medium. The experiments were carried out on 7 dogs under chloroform anesthesia. The vagi were paralyzed by atropin. It was found that intravenous injections of medullary extracts in doses equivalent to 0.005 to 0.010 mgm. of gland substance per kilogram body weight gave pressor reactions of from 20 to 40 mm. Hg. With doses below 0.001 to 0.003 mgm. only depressor effects were ordinarily obtained. Two animals, however, gave only pressor responses. These investigators left open the question whether the change from pressor to depressor reactions was due to the presence of an unrecognized impurity or to the activation of some depressor mechanism.

That adrenal extracts are able to produce vasodilatation and thus a fall of blood pressure was reported by Dale in 1906 (22). This investigator had noted that ergotoxin paralyzes sympathetic fibers that have a stimulating function, leaving more or less intact those having an inhibitory function. A dose of adrenin that, in a normal animal, would evoke rise of pressure was found, after ergotoxin poisoning, to cause a fall. Elliott (23) reported that he had occasionally observed depressor effects from very dilute solutions of adrenin, but apparently was unwilling to accept the observations as valid. Despite such observations,

however, for many years the depressor effect of adrenin was regarded as negligible or non-existent. In 1912 Hoskins and McClure (5), and a year later Cannon and Lyman (24), studied more carefully the systemic reactions to adrenin in different doses. With very slight quantities it was found that the outstanding effect was depression; then as the dosage increased the depressor was gradually superseded by a pressor effect. Cannon and Lyman noted that both the pressor and depressor effects were, within limits, cumulative. They observed, also, that when the initial blood pressure was low, only pressor effects could be elicited. These results were confirmed by Hartman in 1915.

Hartman (25) and his collaborators have more recently published extensive observations on the adrenin vasodilator mechanism. They noted that if the splanchnic nerves were cut, adrenin in doses that had previously caused a rise of pressure now caused only a fall. The part played by the central nervous system in the vasodilator reaction was also studied. A segment of intestine or a limb was cut off from the body circulation and independently perfused with warm oxygenated Ringer's solution. The part was connected with the rest of the body only by its nerves. The organ was then placed in a plethysmograph or the venous outflow was measured directly. Adrenin injection into the general circulation brought about vasodilator effects in the isolated organ, and these must have been mediated by the central nervous system or ganglia. Decerebration failed to affect the reaction. When the medulla was destroyed the systemic depressor reaction was converted to pressor, but the dilator mechanism in both the limb and the intestine was found to be still functional. Destroying the spinal cord failed to abolish the dilator response, hence it was concluded that the effect was partially determined outside the central nervous system, proper. Subsequent investigation showed that local vasodilator effects could be abolished by the destruction of the sympathetic or the dorsal root ganglia. It was found possible, on the other hand, to bring about dilatation by the application of adrenin directly to these ganglia. A systematic study was made of the occurrence of the vasodilator reaction in various species of animals. It was found absent in reptiles, birds and rodentia (rats and rabbits), but present in the opossum; it was demonstrated in the carnivora (cats, dogs and ferrets). But in the cat the mechanism was not functional until about the 11th week of life.

*Factors that modify adrenin reactions.* Various factors have been found to modify the systemic reaction to adrenin. The Goetsch test

for exophthalmic goiter depends upon the augmented sensitivity of the sympathetic system with resulting increase in the response to the drug. Parathyroid extirpation, with its augmented sympathetic irritability, has a similar effect. Simonds (26) found that in dogs subjected to anaphylactic shock and peptone poisoning, the response to adrenin was either much diminished or entirely lost. Schiff and Epstein (27) have reported that general debility in children markedly decreased the reaction to adrenin. Collip (28) found that the pressor reaction was augmented and prolonged by the administration of tissue extracts, whereas the depressor reaction was lost or even converted to a pressor reaction. Similarly, he has shown in a striking way that under identical conditions a given dose of adrenin will cause a depressor response in a dog under light anesthesia, whereas increasing the depth of the anesthesia results in a pressor reaction to the same dose. As is well known, if the anesthesia is made very deep, the pressor effect is finally diminished (29). Cocain somewhat sensitizes the dog to adrenin. Hartman found that a given dose of adrenin which evoked constriction in a limb brought about dilatation when artificial heat was applied. The effects of hemorrhage are sufficiently indicated in Cannon and Lyman's observations that any factor leading to low initial tension converts the depressor to a pressor reaction. Snyder and Campbell (30) perfused adrenalin through the circulation of frogs. It was found that decreasing the acidity of the menstruum increased the vasoconstrictor effect, and vice versa. Collip (31) extended these observations to the dog. He found that the sudden administration by vein of a fairly large dose of sodium carbonate resulted in a change of depressor reactions to pressor. A subsequent injection of acid sodium phosphate was found to convert the pressor to a depressor effect. Such reversals could be obtained repeatedly in the same animal.

From the foregoing it is obvious that there is no justification for classifying adrenin as a vasoconstrictor agent. It is either vasodilator or vasoconstrictor, depending upon the amount used, the organ affected and the conditions of the experiment, as regards body temperature, depth of anesthesia, action of such deleterious factors as tissue extracts, anaphylaxis, etc. A failure to recognize the importance of these modifying factors accounts in part, perhaps, for the existing conflicts in the evidence regarding adrenal pharmacology.

*Effects of adrenin on the heart.* As regards the isolated heart the evidence is strikingly consistent that adrenin causes stimulation. Burridge (32) has shown that a slight amount of adrenin markedly im-

proves the activity of a heart exposed to unbalanced Ringer's solutions. He believes that the adrenin effect is particularly related to the calcium ion effect. With the heart in situ and the vagi intact, the resulting high blood pressure, when larger doses of adrenin are administered, usually leads to secondary depression,—at least of the chronotropic function. On teleological grounds dilatation of the coronary vessels would be expected, and in most animals such results have been obtained. In man, however, Barbour noted that rings cut from the coronary arteries responded to adrenin by contraction only. The problem was further investigated by Barbour and Prince (33), using another primate, the *Macacus rhesus*, the experiments being controlled with rabbits. Isolated hearts were perfused with diluted blood to which hirudin was added. In the rabbits increased coronary outflow always resulted, while in monkeys precisely the opposite effect was seen under all conditions of high or low perfusion pressure, with beating or resting hearts and with all effective doses of adrenin. The doses reported, however, were grossly in excess of any to which the heart of a normal animal is supposedly ever subjected. The problem is in need of further study with regard to the accessory conditions known to influence adrenin reactions and especially with regard to dosage.

*Effects of adrenin upon pulmonary circulation.* The effects of adrenin upon the circulation of the lung have been studied by a number of careful investigators, beginning with Brody and Dixon and Plumier in 1904. The earlier observers ordinarily used quantities at least hundreds or thousands of times as great as those in the normal blood stream. In general, under such conditions, increased pulmonary pressure and vasoconstriction were seen. Tribe (34) perfused the lungs of numerous cats, dogs, rabbits, guinea pigs and ferrets with adrenin in defibrinated blood. With high dilutions, dilatation was obtained, while with large doses, constriction resulted. Schafer and Lim (35) have reviewed the evidence and added many observations of their own. They employed adrenin in dilutions ranging from 1:2,000 to 1:62,000. In nearly every case constriction resulted.

Studies of the effects of epinephrin on the pulmonary circulation in living animals have given varied results. Some observers have reported increased pressure and flow, and others, decreased. Desbouis and Langlois (36) using an electrical method determined the rate of the blood flow in the lungs of the dog. Doses of 0.05 or 1.025 mgm. accelerated the flow; with larger doses retardation was seen. Andres and Cloetta (37) came to the conclusion that adrenin ordinarily has no

effect upon pulmonary circulation. Schafer and Lim (35) studied the simultaneous effects on pulmonary and systemic blood pressure. The results varied somewhat in different animals, but the effects were more or less parallel in the two systems. The sum total of the available evidence indicates that the physiologic effects of adrenin in the lungs are not such as significantly to influence the systemic circulation.

*Effects of adrenin on venous pressure.* Venous pressure, according to most observers, is not greatly affected by adrenin, except in large doses that result in back pressure from the heart. Connet (38) has recently reviewed the literature and reported a somewhat extensive series of experiments upon 50 dogs and 25 cats. She worked always with quantities of adrenin sufficient to cause a rise in arterial pressure. In order to eliminate the effects of respiratory movements and of anesthesia, certain experiments were carried out on curarized and decerebrated animals. In these, such doses as 0.15 mgm. caused marked increase in arterial pressure, leaving pressure in the vena cava unchanged. Ordinarily, however, some increase in the venous pressure was noted, irrespective of whether the heart rate was increased or decreased. Altogether, it would appear that adrenin has relatively little effect upon the veins and this, such as to facilitate the systemic circulation.

From the foregoing data it appears that the adrenals have an important relation to the circulation and it is at least possible that adrenin itself plays a significant rôle. As to whether such is the case under ordinary circumstances, opinion is divided. Some students still tend to the opinion that hypotension resulting from adrenal destruction is due merely to loss of circulating adrenin. Direct attempts to prove this have for the most part given negative results.

IS ADRENIN NORMALLY SECRETED IN SIGNIFICANT QUANTITY? The ideal method of determining whether adrenin is normally secreted in sufficient amount to affect the circulation would be to ascertain the minimal amount that would exert a detectable influence and then to determine whether, under normal conditions, the adrenal glands, together with accessory chromophil tissue, secrete this amount. Up to the present time, however, determinations of the adrenin output under normal conditions have not been made. It would seem that such could be approximately achieved by utilizing Biedl's technique of transplanting the glands to a subcutaneous locus, with care to preserve intact both the circulation and innervation and, later, securing and assaying the efferent blood. With proper allowance for the dilution factor, the amount of adrenin in the blood stream could then be calculated.

The nearest direct approach to the problem has been made by Trendelenberg (39), who withdrew blood from the carotid of the rabbit and passed it through the circulatory channels of the frog. His results indicate that adrenin occurs in the arterial stream at most in a dilution of 1 to 2 or 3 billions. This is a quantity that has no perceptible influence in mammals. Moreover, it was not demonstrated that the minute amount of pressor substance found by Trendelenberg was actually adrenin. This experiment obviously left undetermined whether secreted epinephrin exerts an appreciable influence in the lesser circulation or in the respiratory processes, intervening between the adrenals and the carotid artery.

Observers of a decade ago were of the opinion that adrenin is secreted in fairly high concentrations. Later investigators, however, have found the concentration much less. The method employed in such determinations has been ordinarily to open the body cavity and draw off the blood from the lumbo-adrenal veins, either directly or into a pocket fashioned from the vena cava and assaying it outside the body. Using this method, Stewart and Rogoff (40) and Hoskins and McClure (5) noted in dogs a total output approximating 0.0002 mgm. per kilogram body weight per minute. More recently, Stewart and Rogoff (41) have made a few determinations in dogs, obtaining the blood more directly through a lumbar incision. They secured an output approximately the same as with the older technique. However, when assayed by the pupillary reaction of the experimental animal the output in cats appeared to be about 0.0007 mgm. (42). It is to be noted that in all such cases two factors which probably more or less vitiate the results have been introduced, namely, anesthesia and trauma. The quantity of adrenin necessary to affect blood pressure was found by Hoskins and McClure (5) to be about 0.0005 mgm. per kilogram per minute. Approximately five times as much was required for minimal pressor effects. The determinations of the threshold have been made for the most part in animals under anesthesia, but work in the writer's laboratory, in which the reactions to adrenin were determined under local and under general anesthesia, showed that the thresholds do not vary significantly unless the anesthetic be deep. The data thus obtained indicate roughly that the amount of adrenin secreted is of the general magnitude of that requisite to influence circulation, but the experimental methods have involved too many uncontrolled variables to be convincing.

A number of indirect attacks upon the problem has been made. If secreted adrenin were a normal factor in regulating the circulation it would presumably have a "sustaining" effect. If it be assumed that blood pressure is being continuously sustained by adrenin discharge, the addition of a slightly greater quantity of the drug should result in corresponding increase of pressure. If, however, a dog of quiet disposition be carefully anesthetized with minimal excitement and with minimal trauma and an artery and vein be cannulated, small quantities of adrenin can be introduced into the blood stream with absolutely no effect upon the blood pressure. This observation in itself indicates that no significant amount is being secreted, since an existing threshold has to be passed. As the quantity of injected adrenin is slowly increased, the first reaction to appear is depression. If the infusion continues at a constantly increasing rate, ultimately the depressor effect disappears, to be followed by a pressor effect. The logical conclusion from such observations is that the adrenals are ordinarily quiescent and that the first effective result of adrenin discharge is decrease of arterial pressure, brought about supposedly by dilatation in the skeletal muscle.

A somewhat less convincing experiment is to determine simultaneously the threshold for adrenin vasomotor effects and peristalsis-inhibiting effects. In the writer's experience, peristalsis is brought to a standstill with considerably higher dilutions than are required to exert an effect on circulation. Experiments along this line have been relatively few, however, and have extended over a period of but a few minutes. It is conceivable that adaptation might later occur and intestinal peristalsis be resumed, even while adrenin in pressor quantities was being circulated.

The evidence as a whole militates against the supposition that blood pressure is ordinarily maintained by the stimulating influence of small quantities of adrenin continuously secreted. It is, of course, obvious that all the experiments cited have been made under artificial conditions and hence do not exclude the possibility that in the normal animal, under perfectly normal conditions, such a minimal pressor influence may be exerted but, in the face of the evidence, the burden of proof is upon those who hold this view. A considerable number of clinicians has consistently maintained the view as supporting the theoretical etiology of so-called "hypoadrenalemia." In this connection, it may be recalled that Cannon and Lyman and Collip have reported that with conditions of low blood pressure adrenin in any effective amount exerts a pressor influence. The clinical theory mentioned, if worked out with sufficient

ingenuity, is not, therefore, untenable, although distinctly improbable. The more probable hypothesis to account for the low blood pressure which follows adrenal destruction is that not the medulla but the cortex is at fault. Such a hypothesis must be invoked to account for the final death of an epinephrectomized animal, for it is impossible to maintain life by the artificial administration of adrenin in any way or in any quantity.

**FACTORS CONDITIONING ADRENIN DISCHARGE.** However unlikely it is that adrenal discharge exerts a significant effect upon the circulation under ordinary conditions of quiescent existence, there has accumulated much evidence that under special conditions the rate of discharge may rise to an effective level. The problem has been under almost continuous investigation in the laboratories of Stewart and Cannon for more than a decade and a large proportion of the available data has come from these two sources.

*Nervous control of adrenal discharge.* That stimulation of the splanchnic fibers running to the adrenal glands may lead to augmented adrenin discharge was first observed by Biedl and by Dreyer, who noted that blood collected from the adrenal veins during splanchnic stimulation had a greater pressor effect than that obtained without such stimulation. Recently Tournade and Chabrol (43) have confirmed this by a crossed circulation experiment in which the adrenal blood from one dog was shunted directly to the jugular vein of a second. Splanchnic nerve stimulation in the first gave a rise of blood pressure in the second. Stewart and Rogoff have further shown that section of the splanchnic nerves leads to a marked depression of adrenin output—at least under the artificial conditions of their experiment. The demonstration of functional nerve fibers to an organ is essentially tantamount to proof of its central control.

Langley found that in the cat the splanchnic nerves received white rami from the fourth or fifth thoracic segment to the second lumbar segment of the spinal cord. The immediate source of fibers to the adrenals would, therefore, fall somewhere within these limits. Stewart and Rogoff (44) attempted to locate more precisely the region of outflow by sectioning the spinal cord at various levels. They investigated the adrenal output by the denervated eye reaction of Meltzer and the intestine-uterus method of Stewart. They were unable to detect any difference of adrenin secretion following destruction of any part of the central nervous system as low as the last cervical section. When, however, the cord was further destroyed it was found that se-

cretion ceased and it was concluded that the fibers concerned in the liberation of adrenin do not extend much below the third thoracic segment. They concluded that there is a center for adrenal discharge in the upper part of the thoracic cord. They were unable, however, to demonstrate activation of this center except by pharmacologic means (strychnia or sodium carbonate).

Cannon and Rapport (45) have investigated further the location of the adrenal center. They used as criterion of adrenal discharge, change of the rate of the denervated heart. Careful precautions were observed to eliminate incidental variables such as redistribution of the blood, differences in initial blood pressure, changes in respiration, etc. That the adrenal glands were secreting under the conditions of the experiments was indicated by a decrease in the heart rate when the adrenal glands were removed. It was found possible to bring about an increased heart rate, varying from 12 to 40 beats per minute in various instances, by stimulation of the sciatic or brachial nerves. Successive ablations of the higher portions of the brain failed to abolish this accelerating effect until transection was made about 2 mm. caudad to the corpora quadrigemina. When, however, this portion of the central nervous system was destroyed, sciatic or brachial stimulation no longer evoked increased heart rate. It had previously been shown that removal of the adrenal glands under similar experimental conditions likewise abolished the heart acceleration; hence, it was concluded that the center for adrenal discharge lies in the region mentioned. It was found further that stimulation of the central end of the vagus or of the depressor nerve caused a slowing of the denervated heart by as much as 24 beats per minute. This reaction likewise persisted after ablation of the brain as low as the corpora quadrigemina, but disappeared when the brain stem was destroyed a few millimeters caudad to the corpora. This depression likewise disappeared when the adrenal glands were removed.

*Methods of accelerating adrenal discharge.* The conditions that determine adrenal activity have been under careful study for more than 10 years. As was early recognized, the presence of sympathetic secretory nerves to the glands establishes strong a priori likelihood that adrenal secretion would be brought about by any influence which activates the sympathetic system generally; that is, by such conditions as cause stoppage of gastro-intestinal peristalsis, increased blood pressure, dilatation of the pupils, contraction of the pilo-motor muscles, etc. It has long been known that such reactions are readily evoked by strong

emotions, by pain, and by asphyxia. It is likewise easily demonstrable that they may be evoked by the intravenous injection of adrenin. To prove or disprove, however, to the satisfaction of critical physiologists whether adrenal discharge actually occurs under experimental conditions has presented a very difficult problem. A voluminous controversial literature on the point has accumulated. The evidence, pro and con, can be found for the most part in articles by Stewart and Rogoff and by Cannon and his collaborators. But a few of the observations can be mentioned here.

The deduction that emotions cause adrenal discharge was put to experimental test by Cannon and de la Paz (46) in 1911. By means of a flexible catheter introduced through the femoral vein into the vena cava above the level of the lumbo-adrenal veins, blood was secured from cats both before and after emotional excitation. This blood was then assayed for its adrenin content by use of strips of longitudinal intestinal muscle. Evidence was thus obtained of significant adrenal discharge during emotion. Removal of the adrenal glands abolished the reaction. By essentially the same technique, Cannon and Hoskins (47) obtained evidence of adrenal discharge during asphyxia and strong sensory stimulation. Anrep (48) shortly afterward noted that a denervated limb or kidney first expanded, then contracted sharply, when the central end of the cut sciatic nerve was stimulated. This contraction occurred in the face of an augmented blood pressure, hence could not be regarded as passive. Since the organ was denervated the contraction must have been brought about by some chemical substance. Since the reaction disappeared when the adrenals were excluded the chemical substance was supposed to have been adrenin. Anrep's observation on the denervated limb was confirmed by Pearlman and Vincent (49). These phenomena Stewart and Rogoff undertook to explain on a basis of redistribution of blood or changes in the rate of flow. Burton-Opitz (50), however, as well as other investigators, have recorded evidence indicating that such explanations are not tenable. Gley and Quinquaud (51) studied the effects of asphyxia on adrenal discharge. In brief, they noted that 4 to 8 cc. of adrenal blood caused as great rise of blood pressure when obtained during asphyxia as did 15 cc., obtained without asphyxia. Likewise, they noted that 20 cc. of blood from the vena cava above the lumbo-adrenal veins, after 3 or 4 minutes of asphyxia, caused a rise of arterial pressure from 24 to 45 mm. higher than was produced by the same quantity of caval blood similarly obtained before asphyxia. Similarly Kellaway (52), using the pupillary reaction,

obtained data indicating that asphyxia causes adrenal discharge. More recently Gley has taken the ground that the adrenals do not play a significant part in the experimental vasomotor reactions.

An important advance in technique was introduced by Cannon (53) in 1917. This was the use of the denervated heart as an indicator of adrenal discharge. Stewart had previously used the denervated pupil, but changes in pupillary diameter lend themselves less readily than do changes of heart rate to objective recording. Cannon and Rapport (54) showed that in an animal in which redistribution of the blood was largely eliminated by tying the carotid, brachial and renal arteries and the lower aorta and by cutting the mesenteric nerves, brachial and sciatic stimulation resulted in increased heart rate, varying from 12 to 39, most of the figures falling in the upper range. Such results have been criticised by Stewart and Rogoff on the grounds that it is possible to demonstrate cardiac acceleration, even with the adrenals removed. The explanation of the discrepancy apparently lies in the fact that in animals that are digesting meat there may be discharged from the liver an unknown substance which stimulates the heart (55). If, however, the hepatic nerves are severed, this no longer enters as a disturbing factor, and in such animals clean-cut acceleration of the pulse can be obtained by sensory nerve stimulation and subsequently done away with by the removal of the adrenal glands. That these effects were due essentially to adrenal discharge rather than to circulatory changes of any sort was shown by the injection of adrenalin at a constant rate, simulating normal adrenal discharge, while the sensory nerves were being stimulated. In such cases the augmentation of the heart rate failed to appear; hence it cannot be argued that the tachycardia was due to any sort of redistribution of a constant amount of adrenin coming from the glands.

Recently Cannon and Carraseo-Formiguera (56) have restudied the problem, using Stewart's original technique except that the denervated heart, instead of the denervated pupil, served as test object. They found that when the blood from the adrenal glands was restricted from the circulation, reflex cardio-acceleration was prevented. After removal of the venous block the response again appeared. The time interval between the beginning of reflex stimulation and the cardiac response was approximately the same as that seen after stimulating the splanchnic nerve or injecting adrenin by vein. Similarly, asphyxia for 45 seconds resulted in cardio-acceleration when adrenal egress was possible but failed to do so when the adrenal discharge was blocked.

Altogether, it seems that, despite the ingenuity with which the data have been criticised, enough careful and concordant observations are now available to justify the conclusion that the adrenal glands are controlled by the central nervous system and that they are stimulated to effective secretion, as would be expected, by such influences as affect other organs under sympathetic control. These influences are, especially, strong emotions, pain and asphyxia. The adrenin thus discharged reinforces the sympathetic stimulation leading to a mass shifting of the blood from the "vegetative" organs to those involved in neuromuscular exertion.

#### RÉSUMÉ

The adrenal glands have a definite pharmacologic relation to the circulation by virtue of their production of adrenin. Adrenin causes stimulation of the heart, vasoconstriction in the splanchnic and cutaneous regions and dilatation in the skeletal muscle. Increased or decreased blood pressure may result depending upon dosage and various accessory factors. Adrenal extirpation with its resulting circulatory collapse proves the existence of a physiologic relationship also. This collapse is not entirely if at all due to adrenin lack since it cannot be long forestalled by administration of the drug and it does not ensue when adrenin secretion is reduced below detectable limits. The slow development of the symptoms of epinephrectomy also indicates that they are not due to sudden failure of adrenin as a stimulatory substance. Direct experimentation shows that adrenin often depresses sympathetic irritability. Marked symptomatology develops while the sympathetic system responds well to stimulation. If adrenin deficiency is a factor, it probably operates in the effector rather than the conductor mechanisms. The adrenals are stimulated to secretion by splanchnic nerve irritation, hence are supposedly under central control. Evidence of the existence of an adrenal center immediately caudad to the corpora quadrigemina has been reported. The preponderance of the evidence indicates that adrenal depression is evoked by stimulation of the vagus or depressor nerves and augmentation by asphyxia, pain and emotional excitement. This augmentation results in a mass shifting of the blood from the skin and viscera to the organs involved in neuromuscular exertion. The cortex is probably the indispensable part of the adrenal. The medulla apparently serves merely to reinforce the sympathetic system in times of stress.

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## THE INTERPRETATION OF SPINAL REFLEXES IN TERMS OF PRESENT KNOWLEDGE OF NERVE CONDUCTION

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The contrast between the phenomena of conduction in the peripheral nerve trunk and in the reflex arc has been the theme of much discussion in the literature on the nervous system. The comparative simplicity and approximately machine-like regularity of the response to stimulation in the nerve trunk, as compared with the great complexity of behavior in the animal whose central nervous system is intact, has led to the general view that in the structures of the central gray matter exist functional capacities differing fundamentally from those of the nerve fiber, and indeed having no counterpart in so simple a mechanism as the nerve-muscle preparation. In these obscure functional capacities of the gray matter are supposed to lie the secret not merely of the coördination and adaptation of spinal reflexes, but of volition, memory and habit-formation.

Faced by the vast complexity of the entire central nervous system in even the lowest of vertebrates, we have sought to attack the problems of its physiology by looking for the key to these functions in the spinal reflexes, taking the spinal cord as the simplest mechanism involving the gray matter.

The contrast between nerve-trunk and reflex arc may be viewed from the angle of the diversity of purpose which the structures serve. The nerve fiber apparently exists for the purpose of transmitting messages to remote parts, rapidly, economically and without modification. The central structure appears to serve as a junctional point where messages from many regions may be correlated, relayed and distributed to other regions. In this respect the fibers and centers may be likened to the wires and central offices, respectively, of a telephone system.

Anatomically the nerve fiber is characterized by its comparative simplicity, extending as it does for great distances without branching or changing much in size or form. In contrast with this the gray matter presents a picture of the most prodigious complexity, with intricate end branches and dendrites providing the connections for extensive coördination of conducting paths (1, section II).

Finally, the contrast between nerve-trunk and reflex arc may be viewed from the angle of experimentally observed functional differences. Sherrington in 1906 (2, p. 14) summarized the differences between conduction in nerve-trunk and reflex arc, the most important of which are as follows:—Reflex conduction shows *a*, slower speed as judged by latency of response; *b*, after-discharge, i.e., persistence of response after stimulation has ceased, often for several seconds; *c*, summation, single stimuli in many reflexes failing to produce any response, whereas a repeated series is effective; *d*, irreversibility, conduction from afferent to efferent neurones being possible, but in the reverse direction through the central structure, impossible; *e*, fatigue on continued stimulation, in contrast with the nerve-trunk which exhibits extraordinary resistance to fatigue; *f*, far greater variability of threshold, or ease with which responses can be evoked; *g*, mutual relations between allied or antagonistic reflex arcs, manifesting themselves as reinforcement or inhibition; *h*, far greater dependence on blood supply and oxygen, and correspondingly greater susceptibility to anesthetics. Broadly speaking, these are the most notable differences between the reflex arc and the nerve fiber as regards conduction.

We are confronted with the question; wherein lies the basis for these differences? As already indicated, microscopic anatomy reveals great structural differences; the nerve fiber presents a cylindrical structure surrounded by a membrane, usually by a sheath of myelin, separating the protoplasm within from the body fluids without; the reflex arc, on the other hand, comprises besides relatively simple axons conducting to and from the central structures, an intricate series of end branches whereby the afferent neurone makes contact with the central and motor neurones, elaborately branched dendrites converging in cell bodies containing nuclei, and points of contact or connection between the end branches and the dendrites, little understood as to their finer detail, designated synapses.

Sherrington (2, pp. 15-17) presents cogent reasons for supposing that the nerve-cell body is not the part of the reflex arc to which its peculiar conducting properties are referable, and for looking instead to the synapse, or point of connection between neurones. He suggests that a membrane or surface of separation across which conduction between neurones must occur, might readily account for the functional properties of the synapse.

Loeb (3, pp. 4-6), some years earlier, emphasized the continuity of the conducting protoplasmic path as the essential feature in the reflex arc,

a view which tends to place the burden of its properties rather on the distribution of the pathways than on the presence in them of a transverse membrane of separation between cells.

We might conclude that the indisputable difference between the reflex arc and the nerve-trunk lies in the intricately branching system of interconnections on the one hand, and the isolated, unbranched arrangement on the other. Other significant structural differences are more open to question. In particular, whether there is indeed a true surface of separation between connecting neurones, or whether there is actual protoplasmic continuity, seems not to be settled as yet by any histological research, although the recent work of Marui (4) points to the latter view. So far as I am aware, no thorough attempt has been made to see if other possible attributes of the synapse might provide a basis for its behavior in as satisfactory a manner as the surface of separation to which Sherrington has drawn attention. I shall return to this consideration later.

Most of the literature concerning the physiology of the central nervous system was written before the present concepts of nerve function had been developed. For this reason we find the conducting paths treated as if they were pipe lines through which continuous streams of an imaginary fluid could be poured. Correspondingly, stimulation, as treated in the literature, might be likened to opening a valve or faucet whereby any desired quantity of this fluid is poured into the stimulated nerve. The application of an interrupted current was assumed to start a steady flow of nervous energy along the stimulated nerve, the flow being graded in volume according to the strength of the stimulating current. Then, as the resulting stream of nervous energy encountered various resistances in its path through the central connections, it was supposed to lose its strength little by little in proportion to the resistance encountered (2, p. 156). Little or no attention was paid to the individual nerve impulses making up the stream. The word "excitability" was used in a loose sense without definition, variations in the threshold of stimulus and in the magnitude of response being used indiscriminately as criteria, as if they necessarily had the same meaning. It was natural for physiologists to treat nervous activity in this way, since the apparent gradation of response to stimuli of various strengths, both in the nerve-muscle preparation and in the spinal frog, suggested that the strength of a nerve impulse might be graded as easily as the current flowing in a wire.

Researches in the last fifteen years, especially those of Lucas and Adrian (5), (6), (7), (8), (9), (10), (11), (12) have thrown a flood of new

light on the physiology of the excitable tissues (nerve and muscle), which must necessarily modify our interpretation of the observations, past and future, on the activities of the central nervous system. Specifically there is need of revising all statements which imply without qualification that this system functions by means of continuous streams of nerve energy which may be varied in the manner of streams of water, at least wherever axons are concerned. Also, as Lucas has pointed out (11, p. 48), it behooves us to state what we mean more explicitly than in the past when we speak of excitability.

Even since the firm establishment of the modern concepts concerning the nerve impulse, very few papers dealing with the central nervous system have shown the heed for them which an adequate treatment of the subject demanded. Notable exceptions are Sherrington's recent papers (13), (14), (15) on the flexion reflex and Martin's paper (16) on the vasomotor reflexes, both of these authors having given the most careful consideration to the implications of the new knowledge. Not only do these modern concepts of nerve function place definite restriction upon the events we may suppose to occur in the various axons of the nervous system, but they open to us a vista of great possibilities of drawing in the future a simplified picture of the working of the nervous system. It is conceivable that through a great generalization in this direction, much that is now regarded as quite inaccessible to analysis will become intelligible in relatively simple terms.

The exact nature of the nerve impulse is not yet understood, but such a definite picture of its general nature has emerged from the above-mentioned researches that we may already begin to make far-reaching changes in our interpretation of the nervous system as a whole. The analysis of the functional response in excitable tissues has been carried far enough to indicate a most significant and fundamental similarity in the behavior of all those subjected to quantitative study. Cardiac and skeletal muscle, nerve and the junctional tissue between nerve and muscle, all reveal in their response to stimulation certain essential and fundamental properties in common, differing quantitatively, but not qualitatively. The disturbance which results from stimulation and its mode of initiation both seem to be essentially the same in all. That in tissues so different in structure and embryonic ancestry as those mentioned, these essential elements of function should be the same in all, is a matter of profound significance. It suggests the inherent propensity to react in this way as perhaps a basic property of living cells in general. At all events, it establishes the probability that the pro-

pensity exists in all cells in the nervous system, whose embryonic kinship to each other is far closer than their kinship to muscle.

The establishment of a common, and fairly well understood basis for the execution of the functional response in all those tissues (nervous and muscular) which have yielded to functional analysis, suggested to Lucas the possibility of its universal application, and led him to raise the following question: "Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had a fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation, and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?" (11, p. 2.)

The scope of this idea is large. A reduction of the elements of neural activity underlying consciousness and behavior to the single basis of the nerve impulse, many of whose physical properties are now known, and whose true physical nature may come to be more fully understood in the future, would be a generalization comparable to the reduction of all the various chemical elements to their constituent protons and electrons (cf. 17, p. 348).

**ELEMENTARY FUNCTIONS OF EXCITABLE TISSUE.** Let us now summarize the salient points in the physiology of the excitable tissues as revealed by recent researches. These tissues may be excited by various classes of stimuli, mechanical, thermal, chemical and electrical; of these, electrical stimuli are unique in their ability to excite the tissues without in any way damaging them, and in the ease with which they may be regulated for quantitative study. When an electric current is passed through a portion of a nerve or muscle fiber a local change occurs which is limited to the vicinity of the region through which the current flows. If this local change does not attain a certain critical intensity it produces no remote effects whatever; if it is made sufficiently intense, however, it will set up a disturbance which is conducted away from the point of stimulation over the entire length of the fiber. A fundamental difference exists between the local change and the disturbance which is conducted away from the point of stimulation, a difference which was first elucidated by Adrian and Lucas in 1912 (18, p. 69). To the local change they gave the name "local excitatory process," to the resulting disturbance which sweeps

over the tissue, the name "propagated disturbance," each designation being the most non-committal possible, expressing the most essential features of each. The local excitatory process is distinct from the exciting current, and can be shown experimentally to persist for a time after this has ceased to flow, the rate of subsidence being characteristic for each tissue (19). Its intensity can be graded, depending on the strength and duration of the exciting current. The researches of Nernst, Lapique, Lucas and Hill (20), (21), (22), (23), (24), (7), (25) all point to the view that this local excitatory process consists in a concentration of ions at some point in the tissue. The propagated disturbance which results only when the local excitatory process reaches a certain intensity, sweeps over the tissue, leaving it refractory to further stimulation for a brief interval of time. Normally it obeys the "all-or-nothing" law (9), (10), (26); that is, it cannot be varied in intensity by any variation in the strength of stimulus. The propagated disturbance has been known classically in the case of nerve as the "nerve impulse," in the case of muscle as the "wave of excitation." It is essentially the same sort of disturbance in both tissues, and the single non-committal designation of Lucas is perhaps the best name by which to recognize it wherever it occurs. Following the absolute refractory phase, there is a period of recovery, the relative refractory phase, during which the threshold of excitation is abnormally high, and the size of response which can be evoked is subnormal, both returning gradually to normal. The size of response which can be evoked at different stages during the recovery process may be measured in the case of nerve by either of two criteria, 1, the size of the electric response; 2, the distance the disturbance can travel without extinction through a narcotized region where conduction occurs with a "decrement" (18, p. 95). If the fluid in which the tissue is immersed is slightly acid there is, following the relative refractory phase, an apparently supernormal phase of recovery. During this supernormal phase the excitability and the magnitude of response both become slightly greater than their final resting values (18, pp. 111-114), (27). Adrian has shown (28) that neither the excitability nor the size of response becomes greater than would be found if the same tissue were in neutral fluid. The supernormality is only relative, due to the fact that in an acid medium the final resting stage is subnormal when compared with the condition of the tissue at neutrality.

Perhaps the most important feature of the conception of the nerve impulse which has come from these researches is the fact that the energy of propagation of the disturbance comes not from the stimulus, but from

the fiber itself. It has been likened (11, p. 23) to the burning of a train of gunpowder, in contrast with the transmission of a sound wave whose energy comes entirely from its initiating source. This fact, now well established, should put an end to all efforts to explain the nerve impulse simply as a transient current of electricity conducted along the fiber on the same principle as in an insulated wire; the dynamics of the two modes of conduction are fundamentally different.

The most striking item of evidence that the source of energy is the nerve fiber itself was Adrian's experiment with the interrupted areas of narcosis (9). It was known that a nerve impulse passing through a narcotized region is not extinguished at once, but by a gradual process of decrement. Adrian showed that if the impulse is in this way almost extinguished and then allowed to emerge into a normal region beyond, it recovers at once to its full magnitude. This observation will have an obvious and most important bearing on the physiology of nerve centers, if, as has been suggested, these include regions where conduction occurs with a decrement.

An important exception to the all-or-nothing principle of response in nerve has been shown to occur when the stimulus is applied in a narcotized region which is conducting with a marked degree of decrement (29), (30). Here a certain measure of gradation in the size of response may result from gradation of the strength of stimulus.

One of the most interesting and important features of the functional response in the excitable tissues is the electric response, or action current. This is well known to occur in nerve, skeletal and cardiac muscle and in glandular tissue. It consists of a wave of lowered electrical potential (with respect to inactive portions of the tissue) which sweeps along the excited fiber, marking the progress of the propagated disturbance. Aside from its theoretical interest, it serves as a convenient indicator of function in these tissues, especially in the case of nervous tissue, in which it is the only direct and well-defined objective evidence of activity; it is thus most useful in the study of the nervous system.

The most probable explanation of the various manifestations and attributes of the functional response in excitable tissues is the membrane theory as formulated for the nerve impulse by Brünings (31). This theory holds that the essential element in the structure of the fiber is a semi-permeable membrane surrounding it, which, because it is less permeable to some ions than to others, becomes polarized, i.e., establishes a difference of potential between the inside and the outside of the fiber; and that the nerve impulse consists in a transient breaking down

of this polarization through increase in permeability. An electrical response, such as that just described, would inevitably result from a progressive transient break-down of polarization, and Lillie has offered good reasons for supposing that the action current thus produced is the actual mechanism whereby the disturbance is propagated along the fiber (32). He has shown (33), (34) how an iron wire with a film of oxide on its surface, immersed in nitric acid, furnishes in its behavior a striking counterpart of the nerve impulse in several respects. The analogy must not, however, be followed too far, for whereas the nerve fiber during its relative refractory phase responds with a subnormal impulse, this is conducted without decrement along the fiber (11, p. 39), but the "iron model" when stimulated during its relative refractory phase conducts with a decrement similar to that induced in a nerve by narcosis. This difference probably marks a point of fundamental divergence between the two systems. For a more detailed consideration of the membrane theory reference should be had to Lillie's review in this journal (35) and to a paper by Troland (17) in which there is a suggestive discussion of some theoretical aspects of the membrane theory, but in which the discrimination between fact and speculation is not as clearly emphasized as would be desirable.

As indicated above, certain of the most fundamental properties of the excitable response have been found to be essentially the same in nerve, skeletal and cardiac muscle and the junctional tissue of the nerve-muscle preparation. The established facts in this connection should be more explicitly enumerated. The relation between the strength and duration of an electric current just sufficing to excite the tissues was worked out by Lucas with a high degree of quantitative refinement (5). The duration of the current beyond which further prolongation ceases to reduce further the strength required to excite, is taken as the measure of the rate of subsidence of the local excitatory process. Lucas found (7, p. 245) that under like conditions this duration for different tissues in the frog was as follows:

	<i>Seconds</i>
Cardiac muscle (ventricle).....	2.0
Sartorius muscle.....	0.02
Motor nerve.....	0.003
Neuro-muscular junctional tissue ( $\beta$ -substance).....	0.0009

In spite of the large quantitative differences, the shapes of the curves relating minimal current strength to duration are practically the same for all these tissues. For the sake of more accurate comparisons of

excitation time in different tissues, Lapique (36) has introduced a constant which he calls "chronaxie," the duration of a constant current just sufficing to excite if it has double the strength of the threshold current of unlimited duration.

The all-or-nothing law of response is classical in the case of the heart; it is virtually established for the individual skeletal muscle fiber by the work of Lucas (6) and the later work of Pratt (37); it is well established for nerve by Adrian's experiments (10). The refractory phase, which is an inseparable concomitant of the all-or-nothing type of response, has been measured in the case of cardiac and skeletal muscle and in nerve (38), (39), (40), (41), and the recovery follows the same sort of course in each case; the duration of the refractory phase differs quantitatively in these three tissues in a manner closely proportional to the differences between excitation times given above. The electric response occurs in the same three tissues, having much the same form in all; furthermore, its total duration, rate of increase to maximum and rate of decline bear nearly the same relation in the three tissues as do their refractory phases (42) and excitation times (chronaxies).

Thus far the evidence points strongly to a community of functional propensity in these tissues, the greatest difference between them being one of time relations. So far as I am aware there has been no proof of all-or-nothing response, refractory phase or action current in the neuromuscular junctional tissue, and I believe that such proof would be difficult to obtain.<sup>1</sup> But if we may argue from analogy, we may be justified in supposing it probable that since it resembles the other tissues in its excitatory properties, it also resembles them in the character of its response, and furthermore, that the duration of its response and refractory phase may be approximately three times as brief as those of motor nerve. This must be recognized as conjecture, but it may prove to have an important bearing on the physiology of the reflex arc, as will be indicated later.

It is a familiar fact in physiology that whereas both muscle fibers and neuro-muscular junctions are demonstrably subject to fatigue, nerve fibers respond to repeated stimulation for a long time without suffering in this respect (43). On the other hand, it is known that in absence of oxygen a nerve will cease to function sooner if stimulated than if at rest (11, p. 100). It is possible, therefore, that the difference is one rather of degree than of kind. In emphasizing the common

<sup>1</sup> Bazett's observations (41) appeared to indicate a very brief refractory phase in the junction, but, as he suggested, they may be open to another interpretation.

character of the functional response in all these tissues, there is no intent to belittle the importance of contraction in muscle, an attribute of its response which is absent in nerve. There is no doubt that a wholly new functional capacity is added here, and that it constitutes the muscle's chief function. But this does not alter the fact that in the evolution of function in these tissues there is a fundamental propensity common to all

An important bearing of all this on the physiology of the nervous system as a whole is that in peripheral nerves, at least, nervous energy should not be pictured as flowing in steady streams, but in nerve impulses, each of which is a distinct event. The impulse is transient; it passes on, the tissue is then refractory, and for the next impulse must be excited anew. This conception is established for peripheral nerves; it is most unlikely that it should be otherwise with the medullated fibers in the great tracts within the brain and cord, for morphologically they differ little from the peripheral fibers. Should it be found to apply to all parts of the central conducting path the influence of this conception upon our views of the working of the brain and spinal cord would be profound. Perhaps then, the most significant course of inquiry before us is this: does the type of response portrayed by investigations on the nerve trunk constitute the basis of activity throughout the nervous system? Does every neural disturbance sweep over the conducting paths open to it, leaving them refractory, so that sustained activity must be intermittent? Or is there something at the synapse which can pass into a sustained state of activity uninterrupted by refractory phase, and capable perhaps of gradation?

APPLICATION TO REFLEX PHENOMENA. We may now consider the salient differences between conduction in the reflex arc and in the nerve trunk enumerated above, in the light of the modern concept of the functional response in nerve, and endeavor to see how far we may go, in the present state of our knowledge, along the way indicated by Lucas; that is, the interpretation of reflex phenomena in terms of the principles of conduction as we know it in peripheral tissues.

*Conduction time.* The first point to consider is the slower speed of conduction in the reflex arc, as judged by latency of response. The experiments of Jolly (44) and those of Forbes and Gregg (45) agree in indicating that in the flexion reflex evoked by a single shock, the time consumed within the cord is about  $4\sigma$ . In the case of the knee jerk Jolly found the time to be shorter than this, but in every reflex the rate of conduction, judging from the total distance traversed within the

cord, is much slower than in the peripheral nerve trunk. This delay has its counterpart in the junctional delay in the nerve-muscle preparation. That delay occurs here is a well-known fact (11, p. 66), and if we regard the synapse as a similar junctional tissue, delay in the reflex arc implies no functional property not present in the peripheral conducting path, or at all incompatible with the fundamental principles of this type of response in general. The very much greater delay in the reflex response to weak stimuli, described by Sherrington (2, p. 21), amounting, in the case of the scratch-reflex, to 2 to 3 seconds, involves a different principle, for in these cases the response was only evoked by repeated stimulation. The problem thus becomes one of the summation of propagated disturbances and will be dealt with as such presently.

*After-discharge.* The second point to consider is after-discharge. Sherrington (2, p. 77) has shown in the crossed extension reflex an after-discharge, following the cessation of stimuli, amounting to more than 12 seconds. This is probably in part due to the secondary reflex effect originating in the proprioceptive impulses coming from the extensor muscle itself, but its presence even in the "de-afferented" preparation (46, fig. 16) shows that the discharge of the motor neurones may continue for several seconds after all afferent impulses have ceased to enter the spinal cord. This phenomenon appears at first sight to be at variance with the principle of peripheral conduction in which the disturbance sweeps over the tissue leaving it refractory. There appears to be instead a condition of sustained activity. But when we consider that in the gray matter we are not dealing with isolated, unbranched paths, as in the nerve trunk, but with a complex system in which one afferent fiber is probably connected with many central neurones through extensive branching (1, section II) we see that this sustained activity need not involve any fundamentally different conception of function from that developed in the case of the peripheral tissues. We need only assume sufficiently elaborate and extensive paths including chains of neurones, each adding a considerable measure of synaptic delay, to account for any observed after-discharge without introducing a functional capacity in any way at variance with the all-or-nothing principle. The apparent indications of sustained activity, in which refractory phase plays no part, may thus be due merely to the complexity of the branching paths involved.

*Summation.* The third difference between reflex and nerve-trunk conduction is summation. Superficially the reflex response to a series of stimuli when none occurs to a single stimulus, appears to be essentially

the same phenomenon as the familiar summation of inadequate stimuli in the fresh nerve-muscle preparation. That the two phenomena are fundamentally different was first shown by Adrian and Lucas in 1912 (18), and the distinction is clearly set forth in Lucas' monograph (11, p. 54). The point to bear in mind in this discussion is that whereas in the summation of inadequate stimuli the first stimulus produces only a local effect, no nerve impulse being set up, in reflex summation we are dealing with stimuli which are adequate as far as the afferent nerve is concerned;—each suffices to set up a propagated disturbance (nerve impulse), but the single impulse fails to evoke a reflex response;—a series of impulses is required. This form of summation is therefore designated "summation of propagated disturbances."

Adrian and Lucas (18, p. 72) were able to produce this type of summation in a fatigued nerve-muscle preparation, and to determine the conditions of the conducting path on which it depended. It only occurs when the nerve-muscle junction constitutes a region of decrement sufficient to block a single impulse. It further depends on the timing of the interval between impulses, so that the second shall occur during the "supernormal phase" of recovery following the relative refractory period. Adrian (28) has since shown that this supernormal phase only occurs when the tissue is in an acid medium, but this is a state which normally results in muscular tissue from activity, and therefore presumably accompanies fatigue at the neuro-muscular junction. Adrian and Lucas (18, p. 107) were able to produce in a motor nerve with alcohol vapor an artificial region of decrement, such that a single impulse failed to pass through it, but a second impulse coming in the supernormal phase of recovery, succeeded. Summation of propagated disturbances occurs, then, when there is a region of decrement sufficient to extinguish a single impulse and when a second impulse follows during the supernormal phase of recovery, this condition depending on acidity of the medium surrounding the tissue.

Adrian and Lucas (18, p. 120) pointed out that summation in the reflex arc is clearly a summation of propagated disturbances, and suggested that it might be explained on the same basis as the summation which they analyzed in the fatigued nerve-muscle preparation, the synapse taking the place of the neuro-muscular junction. They state that although the second impulse "may fail to find its way completely through the region of decrement it will have progressed further than the first and so have opened a longer stretch of the conducting tissue to the possibility of supernormal recovery; consequently the third disturbance

will in its turn outrun the second" (18, p. 119). In this way they explain the fact that in some reflex arcs a series of more than two stimuli is required to evoke a response.

Adrian (28, p. 29) has further drawn attention to the fatigability of the reflex arc and its dependence on oxygen as indications that acidity may be a common, if not a constant condition in the neighborhood of the synapse, and that such acidity as occurs there in normal life may suffice to account for both regions of decrement and the supernormal phase of recovery, the two conditions (besides the timing of impulses) requisite for summation.

From this discussion we may conclude that as far as we can see at present, not only does summation as observed in the reflex arc call for no functional propensity differing essentially from those of the nerve-muscle preparation, but that the conditions of variation in the neuromuscular mechanism which enable it to manifest summation of propagated disturbances are such as are altogether likely to exist in the central mechanism as well.

*Irreversibility.* The fourth point to consider is the valve-like action of the synapse whereby impulses can pass from afferent to internuncial or motor neurones, but not in the reverse direction. Sherrington (2, p. 17) suggested that this property might be the result of discontinuity of the protoplasmic path; that the "synaptic membrane," supposed to be interposed between neurones, might be permeable only in one direction to certain ions.

Another suggestion is that of Lillie (32, p. 424), based on his contention that the action current is in itself the essential cause of propagation of the nerve impulse. He suggests that irreversibility depends on the relation between the duration of the action current at one point in the conducting path and the chronaxie of the next point, which must be excited by that action current in order that the disturbance may go on. Let us examine the possibility of applying this idea to the synapse. We may assume, for example, that the time relations of excitation and response in the dendrites which conduct impulses from the synapse to the axon of the next neurone, are much briefer than those of the end branches through which the impulses approach the synapse. In order that conduction should be irreversible, it is only necessary that the action current in the end branch should last long enough to excite the dendrite, but that the action current of the dendrite should be too brief to excite the branches of the afferent neurone, because of their longer

chronaxie.<sup>2</sup> This is, of course, speculative, but it is not at all beyond the realms of possibility, and no new functional propensity would be involved in such a mechanism. It should be noted, incidentally, that this conception does not require any transverse synaptic membrane.

*Fatigue.* The next point is the contrast between the great susceptibility of the reflex arc to fatigue (2, p. 218) and the indefatigability of the nerve trunk. A nerve trunk stimulated 40 or 50 times a second will continue to conduct impulses at this frequency for hours (43), (47). In contrast with this, fatigue in the reflex arc may be readily demonstrated (48).

In the spinal cat with proper controls for electrode polarization the flexion reflex may show marked fatigue in a half-minute or so. That the fatigue is central is proved by the fact that immediately after failure of reflex response stimulation of the motor nerve will evoke a full-sized contraction of the muscle.

Sherrington (2, p. 218) and Lee and Everingham (49) have shown that such reflex fatigue does not involve the motor neurone as a whole, but only the particular channel of approach to it which has been employed. Under Sherrington's direction I found (48) that fatigue of the flexion reflex as evoked through the popliteal nerve usually did not impair the response evoked immediately afterwards through the peroneal nerve, and vice versa. Indeed, the test reflex evoked through one nerve was usually even more vigorous after fatigue of the reflex arc through the other nerve than before, although when the fatiguing stimuli were above a certain strength the subsequent test reflex showed slight impairment. Sherrington concluded from evidence of this sort that the seat of fatigue is in the particular synapse whereby the motor neurone is excited (2, p. 218).

It is well known that in the nerve-muscle preparation the neuro-muscular junction is more subject to fatigue than the muscle fibers. When fatigue is established at this point there is, as we have already seen in connection with the phenomena of summation, conduction with a decrement. Reasons were mentioned in connection with summation for supposing that in this fatigued condition there is a considerable degree of acidity developed in the tissue. Thus, in the case of the nerve-muscle preparation, as well as in the reflex arc, we have reason to suppose that fatigue occurs chiefly in the junctional tissue. In the

<sup>2</sup> Marini's observation (4) that neuro-fibrils may enter a cell body elsewhere than through the dendrites might call for a revision of this scheme, but would not prevent its development in another form.

neuro-muscular junction this fatigue is associated with decrement. Both in connection with summation and, as we shall presently see, in connection with inhibition it has been plausibly argued that an important property of the synapse is conduction with a decrement. If this be so it emphasizes one more point of similarity between the reflex arc and the nerve-muscle preparation (cf. 50), and it will be altogether reasonable to suppose that fatigue acts by increasing the decrement in the synapse as it does in the neuro-muscular junction. Closely associated with the degree of fatigue and the resulting degree of decrement in the synapse, we may expect to find the degree of acidity an important factor in the operation of the reflex arc under various conditions of stress and fatigue.

In this connection, Stiles (51) has drawn attention to the suggestive fact that the finer branches of the neurones in the synaptic region exhibit great attenuation of the conducting substance, and that therefore we should expect any material required for the transmission of the impulse to be more rapidly exhausted here than in the larger fibers. He further remarks, "On the other hand, it should be speedily repaired because it has most extensive surface relations with the surrounding fluids."

The influence of the strength of afferent stimulation in determining its effect on subsequent reflexes, referred to above, presents a puzzling problem. The observations were as follows:

A decerebrate cat was so arranged that an extensor muscle could be reflexly inhibited through either popliteal or peroneal nerve and excited through the opposite sciatic nerve. In most cases inhibition by a series of stimuli lasting 45 seconds applied to the popliteal nerve was found to augment both crossed extension and its reflex inhibition through the peroneal nerve if the prolonged stimulation was below a certain critical strength, but if above this strength, the prolonged stimulation was followed by depression of both excitatory and inhibitory reflexes. The graphic record of such an experiment is shown in figure 1 (48, fig. 14). It may be significant that the critical strength of the stimuli at which this change occurred in the effect on subsequent activity was in two out of three of the animals approximately the same as has since been found just sufficient to excite all the fibers in such a nerve trunk, as judged by the magnitude of the action currents. It is conceivable that for some reason which at present is not clear, prolonged excitation of all the afferent fibers in a nerve produces a generalized synaptic fatigue influencing all the synapses in the center whereby both excitatory and

inhibitory effects are evoked, including those only approached through another allied nerve trunk, whereas if a considerable number of afferent fibers are not stimulated the fatigue is limited to synapses involved by those fibers that are stimulated. On the other hand, it should be noted that the approximation between the strength of stimulus which is maximal for the nerve, and that which is critical as to the central after-effect, is a very rough one at best and may be a mere coincidence. It may be that the whole effect is one of acidity; that a very slight degree of acidity in some way increases the efficiency of conduction, but greater acidity increases the decrement. A few impulses traversing the synap-

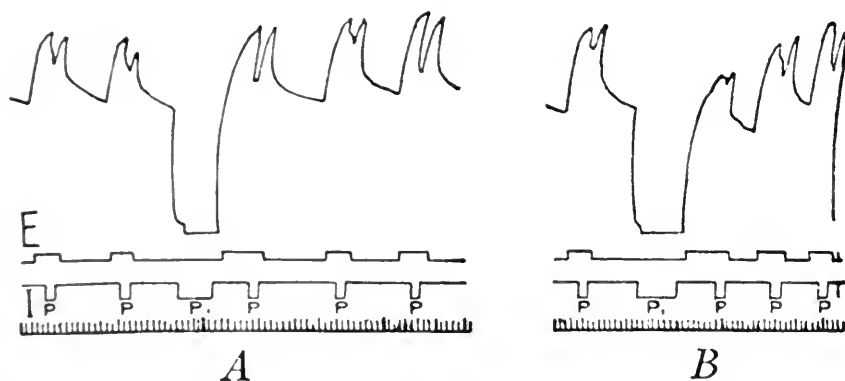


Fig. 1. Vasto-crureus muscle. Ascent of myograph line shows contraction. Rise in upper signal line shows excitatory stimulus (crossed sciatic nerve). Fall in lower signal line shows inhibitory stimulus, marked *P* when applied to peroneal nerve, *P*<sub>1</sub> when applied to popliteal nerve. Time below in seconds. Popliteal stimulation lasted 45 seconds in each case, drum was stopped to save space while stimulation was in progress. In *A*, break shocks to popliteal, 41 Z units; in *B*, 46 Z units.

ses might produce a fatiguing degree of acidity only in their immediate vicinity, thus limiting fatigue to themselves, and facilitating conduction in other adjacent synapses. Activity in a much larger number of synapses, especially if prolonged, might develop enough acid to cause a generalized fatigue of all the synapses in the vicinity. The recent observations of Marni (52) on central fatigue show somewhat generalized changes suggestive of catabolism, and in no way incompatible with the conception suggested above.

*Variability of threshold.* The next point to consider is the greater variability of threshold in the case of the reflex arc than that found in the

isolated nerve trunk. The assumptions as to the degree of variability of threshold in the case of a nerve trunk are generally based on the threshold of the motor nerve as judged by contraction in the innervated muscle. The motor nerve threshold thus determined appears to be far less subject to variation than the so-called threshold of the reflex arc, when stimuli are applied to an afferent nerve. This brings us to one of those points in the traditional physiology of the central nervous system most in need of a searching revision in terms of modern knowledge, and at the same time one of those points in which it is most difficult to see just how the modern conception of the nerve impulse can be reconciled with the empirical facts concerning the central structures. Histologically the afferent nerve fibers do not differ appreciably from motor nerve fibers. Furthermore, it has recently been shown (30) that most if not all of the fibers of a pure sensory nerve in a mammal obey the all-or-nothing law just as do the fibers of the frog's motor nerve. If the stimulus is applied to an afferent nerve trunk how can the threshold of the reflex be other than the threshold of the nerve? If by reflex threshold we mean the threshold of the afferent nerve, why should this show any essential difference, such as greater variability, from the threshold of the motor nerve? It is an empirical fact that the reflex threshold does show this greater variability, and it is difficult at first sight to see how the difference may be explained. The problem is presented most clearly by the experiments of Lutz (53). He showed that in the frog, with electrodes applied to an afferent nerve, the average threshold for the flexion reflex was twice as high as for the muscular response with electrodes similarly applied to the motor nerve. He further found (54) that when the animal was cooled the threshold for the reflex rose nine times as much per degree centigrade as did the threshold of the nerve-muscle preparation. Since he used single shocks of threshold strength, his experiment did not introduce the same opportunity for confusion through summation of propagated disturbances, which enters when repeated stimuli are used. An important conclusion is therefore indicated by his results. If each afferent fiber were connected with a single motor neurone, the pair thus constituting a single isolated reflex arc, it should make no difference in the motor response of that arc how strong a stimulus was used provided it sufficed to excite the afferent fiber. The threshold for the reflex would be simply the threshold for the afferent fiber. In a previous communication (55, p. 285) it was shown that to account for the great variability of reflex threshold compared with that of the nerve trunk, on any basis

assuming isolated reflex paths, would involve a series of coincidences too improbable to be worth considering. Moreover the branched arrangement of the afferent fibers in the gray matter suggests that each one may be connected with many motor neurones, and vice versa. It is therefore misleading to picture a single afferent neurone connected with a single motor neurone as typifying the structure of the reflex arc; we should instead consider the nerve center as a place of convergence and intermingling of many separate paths (1, p. 93). This view is supported by the physiological evidence cited above in connection with synaptic fatigue. The inference is that the convergence of impulses in many afferent fibers in some way produced a different effect in the motor neurones from that produced by a few. The reflex threshold would then depend on the number of afferent fibers excited, and would no longer be identical with the threshold of the most excitable fiber among them. In this way we could readily account for great variations in reflex threshold through agencies which cause little change in the threshold of the afferent fibers.

How can this inferred influence of the number of converging impulses in the nerve center be made to harmonize with our conception of the nerve impulse? It appears to suggest a gradation of central activity incompatible with the all-or-nothing principle. Are we to suppose that at the synapse there is a different sort of activity from the nerve impulse, graded in intensity according to the number of afferent impulses producing it, its ability to excite the motor neurone depending on the intensity to which it is raised? Or are we to look on the synapse as a portion of the conducting path with the same fundamental properties as the nerve fiber, but with different time relations, and perhaps normally conducting with a decrement? Decremental conduction in itself provides the possibility of gradation of intensity in the individual impulse, as has already been mentioned in an earlier section. Yet even without this sort of gradation we may find a basis for explaining the effect of convergence of impulses at a common point in terms of the time relations of the common portion of the path. Forbes and Gregg (56, p. 221) found reasons which will be discussed presently for supposing that some part of the central conducting path has a briefer refractory period than the afferent fibers. A portion of the synaptic region, with a very brief refractory phase, common to all the afferent paths involved, would account for the effect on the basis of summation as outlined above; for even the synchronous volley of impulses set up in the afferent nerve by a single shock might arrive at the common path at slightly different

times, and it would then only be necessary that the refractory phase should be brief enough for some impulses to arrive in the supernormal phase of recovery in order that they might in combination break through a synaptic resistance where without such combination they would be extinguished.

We may conclude that the great variability of the apparent threshold of stimulation in evoking reflex response points strongly to the central intermingling or convergence of conducting paths (evidenced histologically in the branching of fibers) as an essential feature in the interpretation of experimental results. We may further conclude that though these experiments offer difficulties in the way of picturing central activity in terms of the properties of functional response known in peripheral tissues, these difficulties may not be insuperable, possible ways of surmounting them being offered both by the character of decremental conduction and by great brevity of refractory phase somewhere in the central part of the path.

*Mutual relations between allied and antagonistic reflex arcs.* Sherrington (2, p. 175) has pointed out several instances of reinforcement of the activity of one reflex arc by stimulation of an allied arc. For example, flexion of a hind limb is evoked in the spinal mammal either by afferent stimulation of the limb itself or by similar stimulation of the forelimb on the opposite side; combined stimulation of both limbs evokes the response more easily than either stimulus alone. There are several other instances of this sort of reinforcement sometimes referred to as "Bahnung." This phenomenon appears to fall readily into the category of effects just discussed under variation of reflex threshold. If those effects can be explained by convergence of individual afferent paths, so can reinforcement. In this connection may be mentioned Camis' observation (57) that the contraction in the flexion reflex was greater if evoked by simultaneous stimulation of both popliteal and peroneal nerves than could be evoked by stimulating either one alone. This might be ascribed to the connection of the afferent fibers of the two nerves with different motor neurones; but against this view are the facts mentioned in connection with reflex fatigue. The modification (increase or decrease according to strength of stimulus) of subsequent reflex response to stimulation of one nerve following prolonged stimulation of an allied nerve suggests that both sets of afferent paths are connected centrally with the same motor neurones.

We may conclude that central reinforcement is merely additional evidence of convergence of conducting paths from various sources at common points in the nervous system.

A different relation between reflex arcs which is the exact opposite to reinforcement in effect, is reflex inhibition. This is one of the most striking and puzzling of all the phenomena in the physiology of the nervous system. It plays an important part in the "reciprocal innervation of antagonistic muscles" of which Sherrington has made such an extensive study (2, p. 83), (58), (59), (82), (60), (61), (62), (63). The elementary facts of reciprocal innervation in the limb reflexes are as follows: The normal dominant reflex responses to stimulation of an afferent nerve in a hind limb are the flexion reflex and the crossed extension reflex; the flexion reflex consists in reflex excitation of the flexor muscles and inhibition of the extensor muscles in the same limb as the stimulated nerve; the crossed extension reflex consists in reflex excitation of the extensors and inhibition of the flexors in the opposite hind limb. Under certain conditions some of these responses may be replaced by their exact opposites; this may be termed reversal. Several interesting types of reversal have been described; these will be considered later.

Reflex inhibition in its most striking form may best be exhibited in a decerebrate mammal in which all muscles acting on the knee joint except the extensor (*vasto crureus*) are paralyzed by section of their motor nerves. The animal is placed on its back with the femur clamped in vertical position so that the weight of the foot tends to flex the knee. Contraction of the extensor is then shown by the rise of the foot into an extended position, relaxation by its fall. The decerebrate animal will usually exhibit the sustained "tonic" contraction of the extensor muscles known as decerebrate rigidity; thus the knee will be held in an extended position. Failing this, the extensor can be reflexly excited by stimulation of the opposite hind leg ("crossed extension reflex"). In either case, if, while the knee is extended, a strong stimulus is applied to an afferent nerve in the leg under observation, the extensor muscle relaxes, allowing the foot to fall abruptly. This relaxation is part of the flexion reflex which, as Sherrington has pointed out (2, p. 229), is the obvious, defensive reaction whereby an animal in normal life withdraws its foot from a thorn or other object which causes injury. It was once supposed that this inhibition might be effected by nerve impulses going to the muscle by way of special inhibitory fibers in the motor nerve. It has long been established (2, p. 100), (64) that this is not the case; that the seat of inhibition is central, and that the condition for relaxation of the muscle is the cessation of impulses in its motor nerve.

How are we to interpret this striking phenomenon of reflex inhibition in terms of the fundamental properties of the nerve impulse? We

stimulate an afferent nerve and thereby cause impulses to travel to the spinal cord, but by some strange process in the nerve center this activity is transformed into absence of activity in the motor neurones subject to inhibition.

In 1885 Wedensky (65) described a phenomenon in the nerve-muscle preparation, bearing at least a superficial resemblance to reflex inhibition, and in 1911 Lucas, in a brilliant series of experiments, established the true nature of the Wedensky effect (66). Lucas further indicated a possible way in which the principle involved might conceivably apply to reflex inhibition. Much has been written about these points in the literature (26, p. 11), (12), (55), and they are so important that it is worth while to review them here.

The Wedensky effect in the nerve-muscle preparation is as follows: If the preparation is moderately fatigued by stimulation through the motor nerve a stage is reached in which a series of stimuli of proper strength and frequency applied to the nerve evokes only an initial twitch in the muscle followed by its complete relaxation. While these stimuli are being applied, additional stimulation of the nerve nearer the muscle fails to make it contract. If the frequency of the stimuli is decreased to a certain point, it will render them effective in establishing tetanic contraction.

Lucas showed (66), (cf. also (26)) that the explanation of the phenomenon was simply this: The relaxation or "inhibition" only occurs when the neuro-muscular junction is in such a condition (fatigue) that it conducts with a decrement. The frequency of stimulation must be such that each stimulus after the first excites the nerve during its relative refractory phase following the preceding impulse, and therefore evokes a subnormal response. The first impulse of the series, being full-sized, is able to pass through the junctional tissue in spite of the decrement, and therefore evokes the initial twitch observed in the muscle, but the subnormal impulses following the first are all extinguished. Clearly, if the frequency of the impulses is reduced till the nerve is allowed to recover fully from its refractory phase each time, these impulses will no longer be subnormal: they will all be able to pass through the fatigued junction, and tetanic contraction of the muscle will result. This explains why there is a critical frequency of stimulation above which the Wedensky "inhibition" occurs, and below which it is replaced by contraction.

Wedensky suggested that this effect which he described might embody the principle involved in reflex inhibition. Lucas pointed out

(11, p. 93) that we have good reasons for supposing regions of decrement to exist normally in the junctional areas of the central nervous system, and that the only other condition necessary to establish central inhibition on the same basis as in the fatigued nerve-muscle preparation, is that the fibers leading to the region of decrement should transmit impulses with such frequency that each is subnormal, and therefore unable to pass. "A neurone so occupied would certainly be a complete block in the path of any impulses which might attempt to traverse it" (66, p. 88).

Many complications arise when we try to make this conception of reflex inhibition fit all the facts which have come to light in the study of the spinal reflexes (55). We shall consider these complications presently, but for the moment we may conclude the question of inhibition with the observation that the nerve-muscle preparation has functional properties which apparently provide a conceivable basis for explaining reflex inhibition, and that this phenomenon therefore does not necessarily imply any wholly new propensity not existing in the peripheral excitable tissues.

Under the same general heading as reinforcement and inhibition, Sherrington (2, p. 14) mentioned refractory phase and "shock" as occurring in reflex arcs in degrees unknown for nerve trunks. His argument for refractory phase in the reflex arc was based chiefly on the scratch reflex, a characteristically rhythmic series of contractions with a frequency of four or five a second in the cat, evoked by stimulation of the skin (2, p. 45). He showed that an addition to the initial stimulus which started the reflex failed to interrupt the established rhythm, no matter when it was applied. That is, when the flexor muscle was in its relaxation phase no skin stimulus could make it contract until the contraction time arrived according to the established rhythm. More recently Sherrington and Sowton (67) have examined the refractory phase of the flexion reflex and found that it amounts apparently to only  $0.7 \sigma$ . Their method was to send two stimuli into the afferent nerve at various time intervals and find the least interval at which the second stimulus was able to augment the contraction resulting from the first. Clearly a second stimulus falling in the refractory phase of the afferent nerve would fail to set up any second impulse, and would fail to augment the reflex response. It is therefore impossible by this method to measure a reflex refractory phase briefer than that of the afferent fibers. In some recent experiments I have measured the least interval between two stimuli at which the second would evoke a separate

response in a mammalian nerve slightly below normal body temperature, using the action current as evidence, and found it to be approximately  $0.7 \sigma$ . For this reason it is probable that in the experiments of Sherrington and Sowton the limit to the interval was set by the refractory phase of the afferent nerve, and that the refractory phase of the reflex arc was certainly no longer than that of the nerve, and possibly shorter. I have mentioned in connection with reflex threshold that reasons exist for supposing some part of the reflex arc to have a briefer refractory phase than the peripheral nerve fiber. The observations of Sherrington and Sowton are altogether in accord with this view.

The long refractory phase for which Sherrington argues in the case of the scratch reflex, introduces a somewhat different problem. In the first place it involves a wholly different conducting path from the flexion reflex, probably far more complex, and including more internuncial neurones. But more important is the fact that the scratch reflex consists of alternate flexion and extension, and therefore introduces the important factor of inhibition playing its part in the reciprocal relation between the opposed muscle groups. The failure of the flexor muscle to respond to additional skin stimuli during its relaxation phase probably depends on the reflex inhibition of its motor neurones at the moment rather than on a true refractory phase of any part of the conducting path.

The question of spinal shock is one upon which there is great disagreement among different authors. Originally Sherrington stated (2, p. 241) that spinal transection was followed by depression of all reflexes in the regions posterior to the transection, including flexor and extensor reflexes, but that the flexion reflex suffered much less and recovered more quickly than the extensor reflexes (2, p. 248). More recently Sherrington and Sowton have reported (67) that the flexion reflex in response to single shocks is actually increased by spinal transection, its threshold being lower and the contraction with a given stimulus being greater. In some experiments soon to be published, we have found the increase in the flexion reflex produced by single shocks to occur immediately after spinal transection. Sherrington originally mentioned the after-discharge as a feature of the flexion reflex which was especially impaired after spinal transection (2, p. 245). I have seen the flexion reflex in a decerebrate cat changed by low spinal transection from a small contraction with notable after-discharge to a brisk contraction with apparently no more after-discharge than appears in the twitch of an isolated muscle. Before transection, repeated stimuli caused cumu-

lative contraction in a way that they failed to do after transection. It is quite possible, therefore, that the earlier statement about the flexion reflex taking part in the general depression known as spinal shock was due to the fact that it was then customary to use repeated stimuli to evoke all reflexes, and that therefore the cumulative effect resulting from after-discharge before transection led to a larger total contraction with this kind of stimulation than was found when the cumulative effect was abolished by transection. There is no doubt that in this reflex in the mammal the response to single stimuli is increased by spinal transection. The so-called shock effect is in this case not a depression but a modification of the reflex response. It should be noted that in the case of the frog the familiar classroom experiment for demonstrating spinal shock consists in repeated stimuli applied to the skin, which is a very different matter from single shocks applied to a large nerve trunk.

In the case of extensor reflexes there is no doubt that they are depressed by spinal transection. Sherrington has shown (2, p. 243) that this cannot be explained as a lasting inhibition due to irritation by trauma. He argues that it is due to the interruption of certain paths. Pike (68) has urged the view that normally the reflexes subject to shock involve conduction to the brain and back, and that the depression of the reflex is due to the interruption of this path; and further, that the recovery from shock depends on the gradual resumption of function by primitive spinal paths which in the course of phylogenetic development have been superseded by those involving the brain.

This is a difficult matter to settle. But we may note that the muscles whose reflexes are depressed in "shock" are those involved in decerebrate rigidity, a condition which Sherrington has shown to depend on impulses coming from the hind-brain. We may suppose that there is normally within the cord a connection between the afferent fibers from one hind limb and the extensor motor neurones of the opposite hind limb. The ease with which the crossed extension reflex can be evoked in the decerebrate animal as compared with the condition after spinal transection may depend simply on the same principle of convergence of paths which we found necessary to invoke to explain the variations of reflex threshold. On this view the recovery of this reflex from the depression of spinal "shock" would depend on some sort of "canalization" whereby the local connections between afferent and motor neurones in the cord become better able to conduct, and thus less in need of reinforcement through the converging path of descending neurones

from the hind-brain. In short, we might modify Pike's view to the extent of assuming in the intact nervous system a functioning connection between the local afferent and motor neurones, but one with a decrement so great that the summation effect of impulses from the brain is required to overcome it, and further assuming in the gradual change known as recovery from shock, a decrease in the degree of decrement, enabling the same spinal connection to conduct even without this reinforcement.

*Dependence on blood supply and susceptibility to anesthetics.* Reflex centers are strikingly dependent on blood supply as compared with peripheral nerves, or even muscles. Interruption of the blood supply to any part of the central nervous system in the mammal results in rapid loss of function. Inadequate respiration may cause profound derangement in the spinal centers. I have seen in a "pithed" cat (entire brain destroyed) to which the air supplied by artificial respiration was inadequate, abolition of the crossed extension reflex following (and probably because of) an asphyxial convulsion (46, p. 175). In contrast with this it is well known that a mammalian peripheral nerve trunk, dissected away from its scanty blood supply, will continue to respond to stimulation for a considerable time. In some experiments on the conditions of survival of mammalian nerve trunks, soon to be published, I have found an approximately normal electric response in an excised cat's nerve kept in cool Ringer's solution, three days after removal from the animal. On the other hand, a nerve will not survive indefinitely without its blood supply, and it soon ceases to function when placed in an oxygen-free atmosphere.

A concentration of ether in the blood sufficient to abolish all ordinary spinal reflexes does not prevent the stimulation of muscles through their motor nerves. It has been shown (69) that even if ether inhalation is pushed to the point of abolishing respiration and thus causing death, the peripheral nerves will still conduct impulses. On the other hand, ether vapor applied directly to a nerve trunk will soon abolish its ability to conduct impulses, the fibers being subjected to far greater concentration of ether than they are when it is brought from the lungs by the blood stream.

Thus it appears that both in dependence on blood supply and oxygen and in susceptibility to anesthetics, the difference between the reflex arc and the nerve trunk is only one of degree. As in the case of fatigue we may possibly find the key to the difference in the consideration Stiles has brought out, the extreme attenuation of the fibers in synaptic

region and the consequent relatively large extent of surface presented to the surrounding fluids. Both Lillie (70) and Troland (17, p. 341) have mentioned evidence pointing to the view that narcotics such as ether act by changing the permeability of the membrane surrounding the fiber. This view harmonizes well with the suggestion that the extent of surface in proportion to volume is what determines the susceptibility of the synaptic region to narcotics. At all events these differences do not reveal in the center any functional propensity differing essentially from those already examined in the neuro-muscular mechanism.

*Gradation of reflex effect in relation to the strength of stimulus.* Besides the differences between nerve trunk and reflex conduction already enumerated, Sherrington, in the *Integrative Action of the Nervous System*, mentioned the gradation of reflex response when the strength of stimulus is varied. Since that book was written before the all-or-nothing principle had been established, and since Sherrington's recent experiments (13), (14), (15) on this subject overshadow in importance the evidence discussed in the book, I shall pass over the earlier discussion and consider the more recent contributions to the subject. Clearly the all-or-nothing principle must profoundly influence the consideration of the subject, and any discussion of the matter based on the older view of graded nerve impulses has little more than historical interest.

Several researches (15), (81) have dealt with this question in the light of the all-or-nothing principle. The results of these researches have been analyzed and summarized in a recent paper (30), (cf. also (56)), in which the following general conclusions were reached: It is a striking fact that as the strength of afferent stimulation (even in the case of single shocks) is progressively increased, the magnitude of reflex response increases in a way that superficially appears to be incompatible with the all-or-nothing principle. Even after the stimuli have become so strong that the resulting action current in the afferent nerve has reached a limiting maximal value, indicating that all the fibers in the nerve have been excited, the continued increase in the strength of stimulus may result in further increase in the reflex response. The problem arises how increasing the stimulus after all the afferent fibers have been excited can further increase the reflex response. If the size of mechanical contraction of the flexion reflex and the size of action current in the afferent nerve are both plotted against the strength of afferent stimulus, it is found that when the strength is reached at which the action current ceases to increase, the reflex response practically ceases to increase over a wide range of stimulation strengths; in short, the two curves are nearly

parallel. Any further increase in reflex response is usually found to be correlated with a compounding or doubling of response in the afferent nerve and electrical evidence of tetanic response in the muscle (30). The explanation seems to be that when the exciting current (even a single break shock) is strong enough it causes a local excitatory process of such intensity that it outlasts the refractory phase and is able to set up a second impulse in each fiber (56, p. 205). The conclusion is that the only possibility of gradation in the reflex response arises either in the number of afferent fibers excited or in the number of impulses set up in each afferent fiber, this latter factor entering even in the response to a single shock if this is strong enough.

In Sherrington's experiments on the flexion reflex (15) and in one of ours (30) there was evidence that even when the stimulus is not strong enough to set up more than a single volley of impulses in the afferent nerve, it may in some cases evoke in the center a disturbance which results in a repetitive discharge in the motor neurones. Sherrington (15, p. 256) considered this suggestive of a different class of disturbance in the center from the peripheral nerve impulse, a disturbance not subject to the intermittent condition imposed by a refractory phase, but capable of being uniformly sustained. The considerations already mentioned in connection with after-discharge justify us in recognizing that this view is not the necessary consequence of the evidence. The apparent capacity for sustained activity may be due to the complexity of the conducting path. At each point in the system the disturbance may be intermittent, refractory phase following activity, and yet the individual disturbances in the various branches may so overlap in time as to render the sum total of activity continuous.

In spite of the striking extent of gradation in the reflex response, there is nothing in all the evidence which is incompatible with the all-or-nothing law as regards the peripheral nerve impulse, nor is there anything which definitely excludes the same type of disturbance as the basis of central activity.

The extension of the ideas resulting from the analysis of reflex gradation to the problem of sensation has been considered in a previous paper (56, p. 229). It was shown that neither in the auditory nor the optic nerve were there enough fibers to account for the known extent of pitch discrimination in hearing and visual acuity in sight, and the known gradations of intensity of sensation in both, on the assumption that gradation depends on the number of fibers excited. If the all-or-nothing law holds good in these nerve fibers we are forced to seek another

basis for sensory gradation. A possible basis was suggested by the evidence of compound or double stimulation in the peripheral nerves of the leg. An unlimited range of sensory gradation might be based on the frequency with which the impulses follow each other in the sensory fibers. Such a gradation of frequency is what would naturally result if the sensory receptor set up and maintained in the nerve endings a greater or less local excitatory process according to the intensity of stimulation, for a sustained local excitatory process, if intense, would cause the nerve fiber to respond early in the relative refractory period after each previous response, but if weak, it would allow the nerve fiber to recover each time till its threshold had nearly reached the normal level. Thus strong stimulation would set up impulses of high frequency, weak stimulation impulses of low frequency, and between the beginning and the end of the relative refractory phase would be found the range of intervals forming the basis for sensory discrimination of the intensity of peripheral stimulation (cf. 26, p. 386).

*Revision of the doctrine of graded synaptic resistance.* One of the most direct applications of the principles outlined above to reflex phenomena lies in the modification of the concept of graded synaptic resistance (2, p. 155). The old idea was that some synapses presented more resistance to the passage of a nerve impulse than others and, therefore, required stronger peripheral stimulation to enable the impulses to pass through. It was further assumed that if a nerve impulse had to traverse two synapses, the resistances of these would be cumulative. The first of these assumptions is obviously in need of radical revision, since it has been definitely shown that a strong stimulus produces no larger impulses in the individual conducting paths of the peripheral nerve trunk than a weak stimulus. The second assumption, that synaptic resistances are cumulative, is also untenable unless we make the improbable assumption that the axones of the internuncial neurones within the nervous system normally conduct with a decrement through their entire course, and therefore differ profoundly from the morphologically similar axones in peripheral nerves. These points need to be emphasized, for they are still to a considerable extent ignored in the recent literature. The recovery of the nerve impulse on emergence from a region of decrement into a normal region in the conducting path was clearly shown by Adrian ten years ago (9). The bearing of this fact on the doctrine of graded synaptic resistance was explicitly stated in 1915 (56, p. 227), and yet in much more recent literature (71, p. 498) the doctrine is still assumed in its original form as stated above. As

Martin recently pointed out with admirable clearness (16, p. 408), the implication of cumulative synaptic resistance is out of harmony with the all-or-nothing principle, and in particular with the demonstrated fact of recovery of the nerve impulse on emergence from a region of decrement. For if a synapse acts as a region of decrement, it will either extinguish the nerve impulse or fail to do so. If the impulse is not extinguished, but passes into a region of normal nerve fiber beyond, it will regain its full magnitude and be as able to pass the next synapse as if it had never encountered the first one. It is, of course, conceivable though unlikely that all internuncial neurones within the central nervous system do conduct with a decrement. If this were so the resistances encountered in a chain of such neurones would be cumulative, but this essential condition for adherence to the unmodified doctrine of synaptic resistance is not mentioned in those papers in which the doctrine is assumed.

RELATIONS BETWEEN SPINAL REFLEXES: *Experimental facts.* The above survey covers the more general facts concerning reflex conduction, but there are several problems in the relations between the reflex centers controlling antagonistic muscles which call for more detailed consideration. I will first describe briefly the experimental facts, and then endeavor to examine them with respect to their compatibility with the principles suggested by Lucas as a possible basis of reflex phenomena.

Sherrington, in his early work (2, p. 117) on the reflex inhibition of extensor muscles by stimulation of afferent nerves in the same limb, was led to the conclusion that such inhibition was absolute, and could not in any way be overcome by stimulation tending to produce excitatory effects in the extensor motor neurones. Later (59) he made the observation that if the strength of the stimuli applied to the afferent nerve in the opposite leg were chosen with sufficient care a partial inhibition could be demonstrated; that is, combined stimulation produced a degree of muscular contraction intermediate between the full contraction induced by crossed stimulation alone and the total relaxation induced by stimulation of the ipsilateral nerve alone. This effect he called *algebraic summation* of excitation and inhibition. When such balancing or algebraic summation of central effects is produced, the partial contraction of the muscle is usually tremulous (72).

The next point to be noted may be designated *electrical reversal*. The usual effect on the extensor center of stimulating an afferent nerve of the same leg is inhibition. Sherrington and Sowton (73) found that this was regularly produced by strong faradization, but that weak

faradization or, better still, stimulation with an alternating current of 20 cycles a second from a rheonome, produced, instead of inhibition, excitation as shown by contraction in the extensor muscles. Tiedemann (74) found in the frog under strychnine a similar change of central effect from excitation to inhibition on increasing the frequency of afferent stimulation.

Another significant fact is the "*post-inhibitory rebound*." Frequently in the decerebrate preparation exhibiting a moderate degree of tonic contraction (rigidity) in the extensor muscles their reflex inhibition for a few seconds is followed, on cessation of the stimulus, by a "rebound" contraction greater than the tonic contraction existing before the inhibition (75).

Another point is *narcosis reversal*. Sherrington and Sowton showed that under certain conditions a stimulus which normally produced reflex excitation might be made to produce reflex inhibition of the same muscle under the influence of moderate chloroform anesthesia. Similar to this is the fact recently observed (55) that under light ether anesthesia the crossed extension reflex in the decerebrate animal became so modified that during the application of the stimuli the extensor contraction was only partial, but when the stimuli ceased a marked increase in contraction at once occurred, resembling the rebound described above.

Another fact of great interest is the *postural reversal* which has been discussed by Sherrington (62, p. 299) and Magnus (76). In general this means the determination of the type of response to a given stimulus by the limb posture existing when the stimulus is applied. Sherrington has found that certain stimuli will cause flexion if the limb is already passively extended, and extension if it is flexed. Magnus has found that the cat's tail when stimulated at the tip is reflexly drawn toward the median plane from whichever side it happens to be hanging; thus change of initial posture shifts the contraction to the opposite one of a pair of antagonistic muscles.

The next fact to note in this connection is the *reciprocal innervation* of antagonistic muscles. Any afferent stimulus which causes reflex excitation of flexors simultaneously causes reflex inhibition of extensors. Any afferent stimulus which causes reflex excitation of extensors causes reflex inhibition of flexors provided there be any preëxisting contraction of the flexor muscles to inhibit.

Finally, there is the intrinsic tendency of the spinal centers to exhibit *rhythmic alternation* between flexion and extension, under some circumstances, even in absence of afferent stimulation. For instance, Sherring-

ton (77) showed that the scratch reflex proceeds with unaltered rhythm in response to steady cutaneous stimulation of a remote portion of the body when all afferent fibers from the muscles themselves have been cut, showing that the alternations are not conditioned by proprioceptive impulses from the muscles taking part in the act. Graham Brown (78) showed that similar but slower rhythmic alternation (progression rhythm) occurred under a depth of narcosis such that transection of the spinal cord in the lumbar region produced no disturbance in this response, and therefore in absence of afferent stimulation of any sort.

With this summary of facts in mind let us see to what extent they may be interpreted as manifestations of functional responses of the same sort as the peripheral nerve impulse, operating under the complex conditions of the central structures.

*Balancing of antagonistic effects.* In dealing with inhibition, we may consider the Wedensky effect as the most promising prototype, and indeed, so far as I am aware, the only example of a similar phenomenon occurring in peripheral tissues under conditions which have been clarified by a thorough analysis, and explained in terms of the fate of individual nerve impulses. The Wedensky effect has been described and explained in an earlier section. The point to bear in mind now is that in Lucas' proposed application of the principle to reflex inhibition, it is assumed that there is an internuncial neurone through which the impulses must pass to reach the motor neurone, and that because of the decrement at the synapse between the two, there is a critical frequency of impulses in the internuncial neurone above which the effect is inhibitory, and below which, excitatory. For convenience in the discussion I have designated this the "pre-motor" neurone (55).

The fact of "algebraic summation" of central effects, described above, has been mentioned (11, p. 98), (12, p. 45) as an objection to the proposed explanation. For if reflex inhibition is conditioned by a high frequency of impulses in the "pre-motor" neurone, this neurone would be an absolute block in the conduction path as long as the frequency was maintained. If concurrent stimulation of another afferent path leading to this neurone had any effect at all, this should be an increase in the number of impulses traversing it, and this could only increase the certainty of inhibition. The conclusion would be that inhibition is absolute; the excitatory effect should fail to appear in any way. In Lucas' monograph the mention of this difficulty is followed (11, p. 99) by the suggestion that perhaps failure of complete inhibition can only occur when a considerable number of afferent fibers remains unexcited and

therefore a considerable proportion of the motor neurones remains free from the inhibitory effect.

I have examined a number of old records in which the knee extensor was tested with concurrent excitatory and inhibitory stimuli of various strengths, and found that the excitatory effect never broke through inhibition except when the inhibitory afferent stimuli were too weak to excite all the fibers in the nerve (55, p. 287). We may thus dispose of this objection as far as the extensor center is concerned, and conclude that the reflex inhibition which is a part of the flexion reflex, probably does act as a complete block in those neurones in which it is established. In the case of the crossed inhibition of flexors this objection is not so easily met, but we shall presently see that here too there is a possible way of meeting it.

*Electrical reversal.* The electrical reversal has been cited (11, p. 96), (12, p. 44) as supporting the proposed explanation of inhibition. Apparently the facts harmonize well with the hypothesis, for in the Wedensky "inhibition" which furnishes the model, an increase in frequency at the appropriate strength of stimulus, or an increase in strength at the appropriate frequency, would serve to convert excitation into inhibition. Further support is found in the fact that Tiedemann (74), and also Sherrington and Sowton (73, fig. 3) obtained with certain strengths and frequencies of faradization an initial twitch followed by inhibition, just as is found in Wedensky "inhibition."

But further examination reveals a difficulty. The analogy implies that each impulse set up in a given afferent fiber sets up in turn a single impulse in the "pre-motor" neurone, so that the frequency of the afferent impulses is carried through unaltered to this neurone, and there determines whether excitation or inhibition shall result.

The analogy between Wedensky "inhibition" and electrical reversal of reflex effect cannot be applied so simply as this, for Sherrington (63) has shown that a single break shock applied to an afferent nerve, in evoking the flexion reflex, suffices to inhibit the extensors. This might conceivably be due to the use of a shock strong enough to cause double stimulation of the nerve, and therefore a rhythmic series of impulses instead of a single volley. On the other hand, I have evoked reflex inhibition with single shocks which showed no signs of setting up more than single impulses in the afferent nerve when tested with a string galvanometer (55, p. 292). If a single stimulus can cause inhibition, the electrical reversal cannot be explained by the simple extension of the stimulation frequency into the pre-motor neurone. This does

not mean that the proposed explanation of inhibition must be abandoned, but that the requisite frequency cannot depend directly on that of peripheral stimulation; it must be set up in some other way.

In earlier sections of the paper we have repeatedly found reasons for looking to the histologically known branching of afferent fibers as a key to many physiological peculiarities of reflex function. We may find in the resulting convergence of afferent paths at common synaptic points a possible mechanism for the establishment of the necessary impulse frequency in the "pre-motor" neurone. This view would probably imply a much briefer refractory period in that portion of the conducting path where convergence occurred than in a peripheral nerve fiber. But other reasons exist for supposing this to be the case, as has already been mentioned in connection with reflex threshold, and will be discussed more in detail presently. Stimuli too weak to excite all the afferent fibers in the nerve, especially currents of gradual onset from a rheonome, would be more likely than maximal induction shocks to deliver impulses at a given synapse with a slow enough frequency to produce the excitatory effect.

Sherrington and Sowton (73) were unable to produce the ipsilateral extensor contraction (electrical reversal) unless there was an appreciable degree of preëxisting extensor tonus, a condition known to depend on impulses from the hind-brain. This fact seems to be another instance of the need of reinforcement for the overcoming of certain synaptic resistances, similar to that suggested to account for the variability of reflex thresholds. The arrangement of branches and synapses providing for reinforcement by convergence on the one hand, and inhibition by convergence on the other, presents a confusing problem. But such an organization may be pictured, if the branches and dendrites are properly arranged and if sufficient latitude of variation in refractory periods and decrements is allowed (fig. 3).

Sherrington and Sowton suggested that the basis of the electrical reversal might lie in two different kinds of afferent fibers mixed in the stimulated nerve (73, p. 445). This would imply a difference in chronaxie between the two kinds to account for their respective excitabilities by differently timed electrical stimuli. That the reversal occurs in the case of stimuli applied to the internal saphenous nerve which contains no proprioceptive fibers (30), and that Adrian (79) found no evidence of two types of cutaneous sensory fibers with different chronaxies, are facts which are hard to reconcile with this suggestion; yet the possibility must not be overlooked.

*Rebound.* The increased activity of the extensors following their inhibition, is a phenomenon of great interest. The fact that it consists in a transient excess of activity in the extensor motor neurones which before their inhibition were exhibiting "tonic" activity, early suggested the idea that the tonic stream of energy had been pent up by inhibition, and on its release burst forth with renewed vigor (2, p. 212). Aside from the improbability that tonus can be dealt with in this way, the view is untenable (46, p. 160), for Sherrington (75, p. 59) found that the amount of rebound contraction following inhibition shows no correlation with the amount of tonic activity inhibited; for instance, inhibition prolonged beyond a certain time is followed by less rebound than if the inhibition is briefer. Rebound therefore cannot depend merely on the accumulation of the suppressed tonus. It has also been shown that it cannot depend solely on the flexed position of the limb (55, p. 294). Apparently rebound depends on some central effect set up by the same afferent stimulus which during its application causes inhibition.

Sherrington and Sowton, after observing the electrical reversal, suggested (80) that rebound might be due to a twofold reflex influence exerted by the stimulus during its application, the inhibitory being the dominant one, but the excitatory persisting the longer of the two after stimulation ceased.

Let us construe this in terms of our general plan of interpretation. At the outset there is indicated the same assumption made to explain after-discharge, viz., "delay paths," central neurones providing a sufficiently extended system of connected paths to account for the long persistence of activity in the motor neurones after afferent impulses have ceased to enter the cord. In addition we must find a basis for the difference in central effect during and after afferent stimulation. For the inhibition during application of the stimuli we have already assumed the necessary condition,—high frequency of impulses arriving at the pre-motor neurone from many converging paths. To account for the change to central excitation when the stimuli cease, we have merely to assume that the impulses arriving via the "delay paths" alone are below the critical frequency (cf. 55, p. 294). Thus the more direct and densely converging paths producing inhibition and the more circuitous paths through which impulses arrive less frequently, represent the two antagonistic influences exerted in the center.

*Narcosis reversal.* This phenomenon fits readily into the general scheme of interpretation as is shown in Lucas' monograph (11, p. 96).

It is known that lipid-solvent narcotics cause a nerve to conduct with a decrement and that the central portion of the nervous system is far more sensitive to their action than peripheral nerves. Therefore we may expect the decrement of any part of the central conducting path to be greatly intensified by narcotics such as chloroform. If this happened at the terminal synapse a frequency of impulses in the pre-motor neurone which normally was slow enough to be excitatory, would become inhibitory. Thus chloroform would convert excitation into inhibition.

The partial suppression by ether of the crossed extension reflex during application of the stimulus, might similarly be explained by the raising of the decremental block to the critical point in some of the synapses (but not in all), and by a sequence of events similar to that assumed to explain rebound (55, p. 297).

Sherrington has pointed out that under certain conditions synaptic fatigue may serve to convert an excitatory into an inhibitory reflex effect (61, fig. 8), and that this may be explained on the same basis as reversal by chloroform. The indications mentioned above that fatigue induces decremental conduction in junctional tissues are in harmony with this idea.

Just as narcosis reversal may be explained on the basis of increased decrement in the final synapse, so the opposite reversal by strychnine (conversion of inhibition into excitation) (82, p. 288), (83), might be explained on the basis of diminution of decrement. If the decrement in these synapses largely disappears, a frequency of impulses in the pre-motor neurone, normally inhibitory, would become excitatory.

Similar effects of chloroform and strychnine found by Bayliss (84) in the case of vasomotor reflexes are significant in this connection.

*Postural reversal.* Bearing in mind that in general increased convergence of impulses at a pre-motor neurone decreases the chance of excitation of the motor neurones and increases the chance of inhibition, let us assume that shortening of the extensor muscle, active or passive, sets up afferent impulses of a certain frequency, and that these set up impulses in the pre-motor neurones of the extensor center. Sherrington (85), (86) has found that a contracted state passively imposed on an extensor muscle in decerebrate rigidity, is reflexly retained (shortening reaction). This would be explained if the impulses set up in the pre-motor neurones in the manner just suggested were of excitatory frequency. Those afferent stimuli whose central effect is subject to postural reversal may in themselves deliver to the pre-motor neurones in

the extensor center impulses of a frequency also below the critical value. If when such a stimulus is applied the limb is in a flexed posture and no impulses are coming to the pre-motor neurones through the afferent fibers from the extensor muscle itself, then the stimulus will cause an excitatory response, resulting in extension. But if the limb is already extended, so that impulses of an excitatory frequency are already being set up in the pre-motor neurones by the afferent impulses from the muscle, and then if the external stimulus delivers to them additional impulses, we shall have the condition for inhibition, because now the frequency of impulses in these neurones will be raised above the critical value (55, p. 302).

Porter's observation (87), (88) that when one phrenic nerve has been rendered inactive by hemisection of the spinal cord, respiratory impulses can apparently be diverted across the median plane by severing or freezing the opposite phrenic nerve, and thus caused to descend the previously inactive nerve, is at first sight difficult to interpret in terms of our present knowledge of nerve function. It suggests the diversion of a stream by damming up its outlet, a concept altogether at variance with the properties of the nerve impulse. This phenomenon may be simply another case of the postural modification of central effects. The diaphragm and the abdominal muscles operate reciprocally as antagonists. The stoppage of contraction in one half the diaphragm might cause a change in the proprioceptive impulses coming from the abdominal muscles, even if no fibers were left which could bring them from the diaphragm itself, and thus account for the phenomenon on the same principles as the postural reversal of the leg muscles.

*Reciprocal innervation and intrinsic alternation of flexion and extension.* These two topics had best be considered together. Strong afferent stimulation in the hind limb always results in ipsilateral excitation of flexors and inhibition of extensors. This is the flexion reflex and is dominant over all other limb reflexes. Stimulation of an afferent nerve in the opposite leg, if unopposed, evokes excitation of extensors; and if there be any flexor contraction going on, other than that of a maximal flexion reflex, such afferent stimulation inhibits it to some extent (61). The flexors and extensors acting on a single joint never contract fully together. Any stimulus which succeeds in producing extension will at least partly inhibit flexion (60).

We may develop a provisional scheme of connections of the spinal neurones, without claiming for it the least degree of probability in its present form, merely for the purpose of seeing whether it is possible

for any arrangement to fulfil the conditions necessary to explain the phenomena in terms of nerve impulses whose properties are such as I have described. Figure 2 (cf. 55, fig. 7), representing an extensor and a flexor muscle in one limb and the neurones involved in regulating their function, shows a typical afferent nerve fiber entering the cord in that limb, one branch going to the flexor pre-motor, and the other to the extensor pre-motor neurone. Since excitation of the flexors is the dominant reaction, we may assume that the branches approach the flexor pre-motor neurone in such a way that even if all the afferent

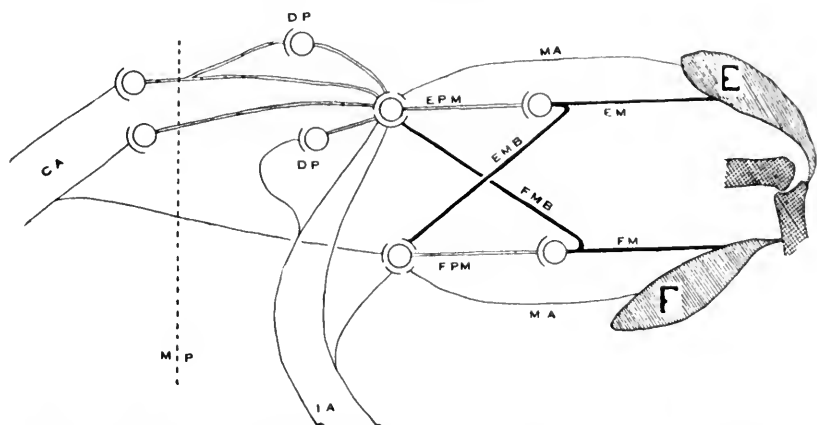


Fig. 2. Diagram of spinal neurones and their connections to provide a possible basis for analysis of reflexes. Afferent neurones shown in light lines; internuncial neurones in double lines; motor neurones in heavy lines. *E*, extensor muscle; *F*, flexor muscle; *MP*, median plane; *CA*, contralateral afferent fibers; *IA*, ipsilateral afferent fibers; *DP*, delay paths, representing extensive central connections to provide for prolonged after-discharge; *EPM*, extensor pre-motor neurone; *FPM*, flexor pre-motor neurone; *EM*, extensor motor neurone; *FM*, flexor motor neurone; *EMB*, collateral branch of extensor motor neurone; *FMB*, collateral branch of flexor motor neurone.

fibers in the nerve are excited they will not set up impulses of inhibitory frequency in the pre-motor neurone, the decrement in the terminal synapse being perhaps relatively slight. The convergence of terminal branches at the extensor pre-motor neurone, on the other hand, is such as to establish an inhibitory frequency in this neurone, provided that all or most of the afferent fibers are excited, and consequently inhibition of the extensor muscle no matter how many impulses may be arriving from other sources. Branches are shown going to other central neurones designated "delay paths" in order to account for the phenomena

of after-discharge and rebound as already described. Paths are shown approaching the extensor pre-motor neurone from afferent nerves in the opposite limb. The arrangement of the branches whereby they approach should be such that the impulses set up in this neurone are below the critical value, and therefore, excitatory in effect.

To account for crossed inhibition of flexors we cannot simply invoke a convergence of impulses at the flexor pre-motor neurone in the same way that we did for inhibition of the extensors, otherwise such inhibition would establish a block which no amount of ipsilateral stimulation would overcome. This would be at variance with the fact that maximal afferent stimulation produces a flexion reflex which cannot be inhibited by any contralateral stimulus. To deal with the crossed inhibition of flexors and to allow for its failure to dominate a strong flexion reflex, we may turn to structures which have been invoked by Graham Brown (78, p. 37) to explain the intrinsic alternation between flexion and extension in absence of afferent stimulation.

Von Lenhossek (89, p. 245), (cf. 1, fig. 161) has described the arrangement of the "side-fibrils" of Golgi, given off from the axons of mammalian spinal motor neurones near their point of passing from the gray into the white matter of the cord. He considered it the function of these side-fibrils to conduct impulses *toward* the axon, but his argument (89, p. 132) is based on anatomical proximity of the ends of the fibrils to the end branches of the "reflex collaterals" of the posterior roots; and this can hardly be taken as proof of "axopetal" conduction. Graham Brown suggested that this side-fibril might be concerned with the function of inhibiting the antagonistic muscle. Impulses set up in a motor neurone would traverse not only the axon but the side branch as well, and through it would reach a point where they could exert an inhibitory effect on the antagonistic motor neurones. To account for the rhythmic alternation he suggested that this inhibitory effect might become fatigued and thus in time enable the inhibited neurone to become active and in turn inhibit its antagonist. In our provisional scheme the side branch leads to the pre-motor neurone of the antagonistic center. The resulting convergence would enable impulses in the motor neurones of one center to raise the frequency of impulses in the antagonistic pre-motor neurone above the critical value, thus establishing inhibition (55, p. 304).

In applying this scheme to spontaneous alternation, we may simply assume that there is a steady stream of impulses from some central source (originating in the cutaneous stimulation in the case of the

scratch reflex, perhaps in a "blood-stimulus" in the case of narcosis progression), and that their arrival at the pre-motor neurones is so timed as to set up in them only an excitatory frequency; whichever motor neurone responds first inhibits its antagonist in the manner just described, until a synaptic fatigue occurs at the point where the side branch acts on the pre-motor neurone; then the impulse frequency in this, falling below the critical value, reverses the process. Since contraction of one of a pair of antagonists is regularly accompanied by inhibition of the other, there is a temptation to seek a simplification of our scheme by relegating all inhibition to the side fibrils (or collateral branches) of the motor neurones. But in so doing we should fail to account for the dominance of the flexion reflex. We must recognize a difference between flexor and extensor centers. This difficulty may be met by retaining the original idea of direct convergence of afferent paths at the pre-motor neurone as a basis of inhibition in the case of the extensor center, and assuming that only in the inhibition of flexors is the activity of the side fibrils essential. The dominance of the flexion reflex would then depend on the fact that strong stimulation, exciting all afferent fibers at once, insures the necessary convergence to inhibit the extensor motor neurones and thus release the flexor center from their inhibitory action.

The question arises, how can the "electrical reversal" occur and ipsilateral extension be evoked in spite of the dominance of the flexion reflex with its twofold tendency to inhibit the extensor center? Why do not the afferent impulses, even if arriving too infrequently to set up extensor inhibition by direct convergence, induce a flexion reflex and thus inhibit the extensor by virtue of the side fibrils? The answer to this question may be found in the consideration that whereas our diagram shows but one neurone of each type, actually in the spinal cord, there are thousands, and each motor neurone may send branches not to one antagonistic pre-motor neurone, but to many. The extensor center is characterized by a slower type of response, and especially by a longer after-discharge, than the flexor center (2, p. 30). In our scheme of analysis this means that its motor neurones are approached through a more extensive series of "delay paths." This arrangement may include more extensive connection for each afferent fiber, so that a small number of afferent fibers will reach the entire coördinated group of central neurones, whereas with less extensive branching in that direction a large number of afferent fibers is required to reach all the flexor motor neurones. Stimulation of an afferent nerve with the kind of weak

stimuli which evoke ipsilateral extension, although exciting only a few afferent fibers and through them only a few flexor motor neurones, may through the extensiveness of the delay paths set up a generalized disturbance throughout the extensor center. Then even if a few isolated flexor motor neurones have been excited, they will soon be inhibited through the side fibrils of the much larger number of extensor motor neurones in action.

In like manner we may deal with the crossed inhibition of flexor muscles. Although a flexion reflex induced by stimulation strong enough to insure excitation of all fibers in the afferent nerve apparently cannot be inhibited by any contralateral stimulation, Sherrington and Sowton have shown that a flexion reflex evoked by moderate stimulation may be so inhibited (61). We may suppose that if a sufficiently large number of afferent fibers is not excited there may be a number of extensor pre-motor neurones not subjected to the inhibitory effect. Then even if enough flexor motor neurones are excited to cause a substantial flexor contraction (as in the experiments of Sherrington and Sowton), the extensor motor neurones which are called into action will, by virtue of their side fibrils, inhibit enough of them to cause a demonstrable decrease in the contraction.

These considerations together with the synaptic fatigue invoked to account for spontaneous alternation of flexion and extension, may also account for the rhythmic alternations induced by concurrent excitation and inhibition in the extensor center (72). First one group of neurones will gain the ascendancy, and then lose it through synaptic fatigue at the ends of the side fibrils.

An apparent objection to the scheme just described arises from the recent experiments of Olmsted and Warner (90). They found that when the contractions and relaxations of the antagonistic knee muscles in the decerebrate cat were registered together on a rapid drum, the latency of relaxation in one muscle was regularly longer than that of contraction in its antagonist. Extensor relaxation began  $14\sigma$  after flexor contraction; flexor relaxation  $19\sigma$  after extensor contraction. In the scheme outlined above we found it necessary to assume that if the extensor motor neurones were active when a stimulus was applied to an afferent nerve the flexor muscles could not contract until their motor neurones had been released from the inhibitory action of the extensor motor neurones by the inhibition of these through direct convergence of impulses at their pre-motor neurones. This view appears at first sight to be incompatible with the observations of Olmsted and Warner;

apparently inhibition of extensors should follow excitation of flexors, not precede it. This objection may be readily answered by a consideration of the contraction time of a muscle. Inhibition consists in the stoppage of impulses from traversing the motor neurones. Suppose when a flexion reflex is evoked the last impulse to traverse the extensor motor neurone and the first to traverse the flexor motor neurone occurred at the same moment, and suppose the conduction time the same in the two motor nerves. Then the latency of extensor relaxation would be longer than that of flexor contraction by the time elapsing between the initiation of the last propagated disturbance in the extensor muscle and the commencement of relaxation following it. Judging from myograph records of the simple twitch (91, p. 25), the time required for a muscle to pass the maximum of its contraction at the mammalian body temperature would be about  $15\sigma$ , approximately the difference noted by Olmsted and Warner. The motor nerves are nearly the same length and their conduction times probably differ by very little; the speed of nerve reactions is so great and the distances involved in the spinal cord so small that the whole chain of processes involved in the release of the flexor motor neurones from inhibition in the manner suggested would probably occur easily within the reduced reflex time,  $4\sigma$ , already noted for the flexion reflex. Therefore, the difference due to the contraction time of the muscle is so large that it completely obscures any small difference in time which may elapse between cessation of extensor motor impulses and commencement of impulses to the flexors. There is, therefore, no real objection raised by the observed difference in latencies.

It cannot be too strongly emphasized that the particular arrangement of neurones shown in the diagram is not for a moment supposed to represent the actual arrangement of neurones in the spinal cord. It is developed simply for the purpose of seeing whether any conceivable arrangement might account for the reflex phenomena without postulating new and unknown functional properties. As we shall presently see, another and quite different arrangement may be developed to answer the same purpose.

**MOTOR INNERVATION IN TONUS AND OTHER SUSTAINED CONTRACTION.** The frequency of nerve impulses involved in most of the activities of the nervous system on which evidence has been obtained is higher than has been generally supposed. The appearance of a characteristic action current frequency of about 50 per second in human muscles in voluntary contraction led Piper (92) to conclude that this was the frequency of impulses in the motor nerve fibers innervating these muscles. Follow-

ing the lead of Buchanan (93) we have found evidence (94) indicating that the nerve-impulse frequency in voluntary innervation of the forearm flexors is much higher than that appearing in the muscular action currents. The true frequency seems to be not less than 300 per second.

In contrast with this finding is the recent observation by Gasser and Newcomer (95), confirming those of Dittler (96) and Garten (97), that in the dog the action currents in the diaphragm correspond exactly with those of the phrenic nerve simultaneously recorded, the frequency being about 100 per second. It is interesting that the evidence should indicate such a difference in the mode of innervation of two muscles, both under voluntary control. No reason for the difference is known, so far as I am aware.

The known refractory phase of medullated nerves at mammalian body temperature is such that it should set an upper limit to their nerve-impulse frequency in the neighborhood of a thousand per second. If the internuncial neurones do not differ greatly from the peripheral nerves we should expect to find the critical frequency in the assumed pre-motor neurone somewhere between 1000 and 300 a second, judging from the observed duration of the relative refractory phase in motor nerves.

The requirements of the proposed scheme suggest a briefer rather than a longer refractory phase in the pre-motor neurone than is found in peripheral nerves; therefore when reflex excitation occurs the disturbances should be available for exciting the motor neurone at least as often as it is able to respond. Therefore we should expect the frequency of impulses in the motor nerve to lie somewhere between the limits mentioned, viz., between 300 and 1000 per second. This conclusion is in harmony with that arrived at by experiment in the case of human forearm flexors in voluntary contraction.

*Tonus.* Tonus is a baffling phenomenon, especially when one attempts to interpret it in terms of propagated disturbances in the tissue which exhibits it. It is a well-known fact, which has often been cited (98), (99, p. 535), (100) in connection with the innervation of skeletal muscles, that in the mollusc, pecten, there are two distinct types of muscle, one capable of executing movement, the other a "postural" muscle apparently not concerned with movement, but capable of maintaining its contracted state against great force without expenditure of energy, thus dynamically resembling a vise. Sherrington (98) has noted the striking similarity of behavior between the extensor muscles in moderate degrees of decerebrate rigidity and the postural muscle of

pecten. The condition he describes is one that he calls "plastic tonus." The muscle assumes any degree of shortening passively imposed on it, and maintains the posture for a long time, apparently without fatigue. Roaf (101) found no evidence of increased metabolism in muscles during decerebrate rigidity; and Bayliss (102), measuring heat production, found evidence of great economy of energy in this condition. On the strength of these data Wilson (100, p. 558) states that "the evidence of duality of muscle function in vertebrates appears satisfactorily demonstrated." (cf. Kahn (112) and Einthoven (113)).

What can be the basis of such a duality of function? We know that the state of decerebrate rigidity depends on nerve impulses, both afferent and efferent; it is a reflex. Cutting either the afferent fibers from an extensor muscle or the motor fibers innervating it abolishes the "tonus" (2, p. 301). Some influence passing along the motor nerve must maintain it. The only influence we know of that can traverse a nerve is the nerve impulse or propagated disturbance whose properties I have described—a definite, transient change, giving no evidence of qualitative variations. The nerve impulse is what calls the muscle into action when the nerve is artificially stimulated; it is supposed to be the means of evoking voluntary and ordinary reflex contractions in the muscle. But "tonus" is supposed to be evoked by something different. Are we to assume that the nerve is capable of transmitting another wholly different kind of influence of a character as yet unknown? Or may we yet find a basis for explaining "tonus" as well as other reflex acts in terms of the well-recognized propagated disturbance?

Buytendyk (103) has shown that in decerebrate rigidity action currents may be led off from the extensor muscles showing practically the same frequency as those of voluntary contraction, and varying in amplitude rather than in frequency in various degrees of rigidity. This result tends to indicate that reflex "tonus" is not fundamentally different, as regards the underlying type of disturbance, from voluntary and other reflex contractions. Experiments, of which a preliminary report has appeared (104), fail to show other than a quantitative difference between the crossed extension reflex, post-inhibitory rebound and the tonic contraction of decerebrate rigidity, as regards motor innervation. They do not, however, prove definitely that qualitative differences may not exist.

There is a conceivable basis for explaining the extraordinary economy of energy in reflex tonus as compared with voluntary contraction, arising from the explanation of the "shortening reaction" suggested above.

Barbour and Stiles (105) have found evidence indicating that sustained reflex contraction sometimes consists in alternate periods of activity and rest in individual muscle groups. Possibly a group of fibers in a shortened state, either by virtue of their own contraction or of those about them, sends to a limited number of motor neurones the requisite proprioceptive impulses to establish reflex contraction. The muscle fibers thus excited may be different from the first group. Synaptic fatigue (perhaps at the junction of the afferent fibers with the pre-motor neurones) releases these muscle fibers before they become subject to fatigue, but meanwhile their contraction has reflexly evoked that of a third group. And so the fiber groups may take up the load in rotation and, for some reason, by this means attain an economy otherwise impossible (cf. 111).

It is obviously unwarrantable to consider the evidence at hand sufficient to establish such a mechanism of rotation as that just described; it is merely mentioned to show how many possibilities arise in a system so complex as we know the neuro-muscular mechanism to be. The object is to show that the same kind of propagated disturbance which has become familiar in isolated nerve and muscle, may be the basis of the baffling phenomena of reflex tonus, the apparently fundamental differences in the kinds of activity observed depending in reality on the vast complexity of organization in the system as a whole. The conclusion to be drawn is that in spite of the striking resemblance between plastic tonus in skeletal muscles and the postural or "catch" muscle of pecten, we are not justified, without further proof, in assuming it to depend on a function in muscle distinct from that involved in "tetanus," evoked in turn by a special function in nerve distinct from the ordinary nerve impulse.

Parker has suggested that the possible function of the neuro-fibrils has been somewhat overlooked in physiological discussion, and that conceivably these may be concerned with the transmission of such special impulses as may induce tonus. This interesting suggestion is one which is difficult to approach by experiment, but possibly it finds support in the observation of Lucas (27) that the nerve in the crayfish claw contains two "excitable substances" of widely different chronaxie.

The theory that tonus in skeletal muscle depends on innervation through the sympathetic nervous system (114) has been shown to be without adequate foundation (115), cf. (116).

A PHYSICAL VIEW OF FUNCTIONAL DIFFERENCES. Looking at the nerve impulse from the point of view of the physical properties of the

fiber, we may find considerations which possibly will clarify the problems of reflex conduction. Reasons have already been given for concluding that essential features in the conducting function of the nerve fiber are the polarized state of the membrane at rest, and the breaking down of this polarization (difference of potential inside and outside) in activity, the depolarization being propagated by means of the resulting action current. Lillie (32), comparing different nerves and muscles, and Lucas (106), observing a muscle at different temperatures, found cogent reasons for concluding that the velocity of conduction depends on the speed of development of the electric response at a given point in the fiber. Lillie reinforced this conclusion with measurements showing that the action current in nerve is just about strong enough to account for the actual velocity of the impulse on this basis (32, p. 434).

Williams and Crehore (107) attempted to explain the nerve impulse as a transient electric current conducted as in a cable. Their explanation as originally formulated proved untenable for reasons which have already been mentioned (p. 367). But certain facts were brought to light in their investigation, which must have an important bearing on the problem. They found that the distributed capacity of a motor nerve fiber as estimated from its histological structure and the known chemical properties of the myelin sheath, together with the ohmic resistance estimated from measurements of a whole nerve, suffice to account for the transmission of a transient electrical current in such a conductor as the fiber appears to be, at the same velocity as is actually found in the nerve impulse. The agreement is remarkably close; furthermore the difference between the velocity of conduction in medullated and non-medullated nerves is the same sort of difference that they would expect on theoretical grounds because of the greater capacity involved in the case of the thinner sheath.

At first sight Lillie's view that velocity of conduction depends on the rate of development of the action current appears to place the cause of the velocity in something quite distinct from the physical constants, resistance and distributed capacity; the two views seem to have nothing in common. But when we consider what the necessary effect of resistance and capacity must be on such a conductor we see that we must deal with the two ideas together. The question arises, what determines the speed with which the action current develops at a given point in the fiber? Suppose the speed of conduction does depend on the rate of rise of the action current, can this electrical disturbance be conducted along such a conductor faster than the capacity and resistance would

permit the crest of a transient current introduced from an outside source to travel? Both resistance and distributed capacity retard the progress of a transient change of potential imparted to an insulated conductor. The structure of the nerve fiber is such that there must be resistance and distributed capacity in it of the general order estimated by Williams and Crehore. These physical constants must act in the nerve as in a cable, retarding the development of a potential change at any given point, and therefore retarding the velocity of transmission. Thus the rate of rise of the action current and the correlated velocity of conduction must together be controlled by these physical constants. On this view one function at least of the relatively thick myelin sheath in a medullated fiber would be to insure high speed of conduction, a result of obvious utility in the rapid coördination of movement.

In emphasizing the community of functional capacity in all the excitable tissues, I called attention to the approximate agreement between quantitative variations in the duration of the different aspects of function—chronaxic, action current, and refractory phase. Turning to the structural differences in the known tissues we may look for a basis of these differences in the dimensions of the different structures which must determine in part the resistance and capacity, and we may further attempt to correlate velocity of conduction with the other quantities on the same basis. Besides these constants we must consider also the ease with which excitation occurs, i.e., the intensity of the action current which must occur at a given point in advance of the crest of the approaching wave in order to excite the tissue there. In general we find that those tissues which are rapid in one aspect of their function, tend to be rapid in others. Muscle, as compared with nerve, has a long chronaxic, long action current, long refractory phase and slow conduction. The thin sheath and consequently large capacity might well account for this. The comparison of medullated and non-medullated nerve fibers shows similar differences. On the other hand, Adrian (42) has shown a lack of parallelism between nerve and muscle in the comparison of refractory phase and action-current duration. If we knew the three determining factors,—resistance, capacity and local excitability in each case we might find in them the basis for this divergence.

From the point of view of reflex function it will be of interest to see if these concepts can throw any light on the properties of the attenuated portions of the nerve fibers in the synaptic regions. We have already seen several functional properties wherein synapses resemble the junctional tissue in the neuro-muscular mechanism. It would go far toward

removing functional characters from the realm of the unknown if we could find a probable basis for them in the dimensions and resulting physical constants of the structures involved.

The nerve-muscle junction has been shown to have the briefest chronaxie of all the tissues examined. I have already mentioned Bazett's observations which pointed to a correspondingly brief refractory phase in this tissue, and the likelihood of a correspondingly brief duration of response. Its speed of conduction, however, instead of being rapid, as we might infer by analogy, is slow compared with nerve conduction, for a delay in transmission is demonstrable there. We do not know its finer structure well enough to seek an explanation of these facts in terms of the probable physical constants.

In making a comparison with the synapse we may start from the observed facts indicating many physiological resemblances between it and the neuro-muscular junction,—decrement, fatigue, delay, etc. Forbes and Gregg were led by their observations (56, p. 221) to infer that in some central part of the reflex path the refractory phase was briefer than in the peripheral afferent nerve fiber. This inference seemed necessary to account for summation of propagated disturbances on which apparently depend the observed variations of reflex response to graded afferent stimuli. This idea fits in well with the other apparent similarities between the synapse and the neuro-muscular junction. We do not know the structure of the synapse in sufficient detail to apply physical principles to conduction in it with much confidence of accuracy, but we may attempt a trial hypothesis based on such properties as are histologically apparent. One point we are sure of,—great attenuation of the finer terminal branches and of branches of the dendrites. The question of contact or protoplasmic continuity between neurones has been much debated. Sherrington (2, p. 16) has been inclined to ascribe most properties of the synapse to a synaptic membrane which may be looked on as transverse, i.e., interrupting the continuity of the conducting path. Marui (4) maintains that the neurofibrils of one neurone pass directly into the cell body of the next. It has already been noted that Lillie's explanation of irreversible conduction at the synapse does not require a transverse membrane (32). Conceivably neurones, though arising as separate cells, establish protoplasmic fusion in the development of functional reflex arcs, possibly by a process similar to the formation of foreign body giant cells (108). From the point of view of the membrane theory of nerve conduction it is easier to picture transmission of the impulse through a reflex path if we assume the membrane surrounding

the conducting unit to be continuous, and not interrupted by a transverse membrane. It will be of interest to see if the properties of the synapse could depend on variations in the dimensions and the resulting time relations of different portions of the path, rather than on a transverse membrane, especially in view of the doubt cast on the existence of such a structure by Marui.

Applying the principle of physical constants to the synaptic region we may be certain that one result of attenuation will be a great increase in the ohmic resistance per millimeter. This will tend to retard conduction. The distributed capacity will depend on the ratio of internal to external diameter; a thin sheath in proportion to the total diameter of the fiber confers a large capacity. So far as I know, data are not available for estimating whether capacity would undergo increase or decrease as the fiber splits into its finer branches. Small capacity would tend to enable the electric response to develop rapidly at a given point. Therefore if the sheath continues to be as thick in proportion to the total fiber as in peripheral nerve, conditions may exist for a brief duration of response, and yet conduction would be relatively slow because of the high resistance. We do not know what determines the duration of refractory phase, but in general it shows fair correlation with duration of response (42); furthermore we should expect the exhaustion and renewal of material, such as might determine the refractory phase, to occur more rapidly in a small structure than in a larger one (51). Therefore there is some likelihood on various grounds that the fine end branches may be characterized by brief chronaxie, response and refractory phase as compared with the larger peripheral fibers. Table 1 shows the approximate dimensions and other data bearing on the possible influence of these dimensions upon function. The dimensions are taken from Lewis and Stöhr (109), the times are roughly estimated for temperatures between 15° and 20°C., from the data of Lucas (5), Lapicque and Legendre (110), (cf. 99, p. 401), Lillie (32) and Adrian (42).

The next question is,—what will happen if a nerve impulse conducted in accordance with the principles assumed, encounters a region where the fiber splits into a number of finer branches? According to the principle of Lillie which we are at present assuming, the electric disturbance in the last part of the full-sized fiber is the cause of excitation in the smaller branch; if this electric disturbance is of such long duration that it continues to be above threshold value till after the response and the ensuing refractory phase in the more rapidly acting branch, it will set up a second response in the latter; if the branch has a brief enough

refractory phase it may respond thus three or four times to the action current in the main fiber. Thus a single nerve impulse entering the terminal branches may conceivably be broken up into a series of briefer impulses. Conceivably this phenomenon occurs at the neuro-muscular junction, and may provide a basis for explaining the increased delay in conduction of an early second response from nerve to muscle, described by Lucas (39). Experiments are now in progress bearing on this point. They do not go far toward revealing whether this phenomenon occurs or not, but at present the results are in harmony with the hypothesis.

Let us see if such a mechanism can form a basis for explaining the various reflex phenomena, and thus enable us to dispense with the confusing transverse membrane, and possibly also to simplify the

TABLE 1

TISSUE	SKELETAL MUSCLE	MEDUL- LATED NERVE	NON-MED- ULLATED NERVE	$\beta$ -SUB- STANCE	HYPO- THETICAL SYNAPSE
Total diameter of fiber.....	30 $\mu$	20 $\mu$	3 $\mu$	?	?
Sheath.....	Thin	Thick	Thin	?	?
Resistance.....	Low	High	High	?	Very high
Capacity.....	Large	Small	Large	?	?
Chronaxie.....	4.0 $\sigma$	0.3 $\sigma$	20.0 $\sigma$	0.04 $\sigma$	?
Rise of action current to maximum.....	2.5 $\sigma$	0.5 $\sigma$	50.0 $\sigma$	?	?
Velocity of conduction (meters per second).....	1.5	30.0	0.2	Slow	Slow
Absolute refractory period....	4.0 $\sigma$	2.0 $\sigma$	?	Brief?	Brief?

arrangement of neurones. For example, we may assign different frequencies of response to various positions of the dendrites, and include in them the necessary regions of decrement, thus dispensing with the "pre-motor" neurone altogether.

In order to illustrate how this idea may be applied, let us consider a specific problem mentioned in connection with electrical reversal and its dependence on tonus, viz., the arrangement of branches providing for reinforcement by convergence of impulses at one point and inhibition by impulses approaching from another source. Let figure 3 represent a motor neurone with the converging branches of its dendrites, each in connection with an afferent or internuncial fiber; let *A* and *B* be the paths whose impulses reinforce each other on the principle of summation; let *D* be the path through which inhibitory impulses arrive.

The decrement in *C*, where *A* and *B* meet is such that a single impulse fails to pass; the refractory phase there is very brief. It frequently happens that impulses arrive through *A* and *B* at the right interval to cause summation, the second falling in the supernormal phase of recovery in *C*. A second impulse so timed will pass the remainder of the dendrite and set up an impulse in the axon *F*. Any impulse approaching through the afferent fiber *D*, when reaching *d*, the most attenuated part of that path, will be broken up into a series of very brief impulses whose frequency is so high that each reaches the larger main trunk of the dendrite *E* early in its relative refractory phase following the preceding impulse, therefore the disturbances in *E* will be subnormal and fail to pass such decrement as exists in *E*. Thus as long as impulses approach through *D*, *E* is the seat of a total block to impulses coming from all branches of the dendrite.

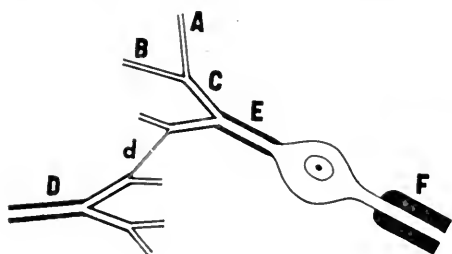


Fig. 3. Diagram of motor neurone with hypothetical arrangement of conducting paths. See text.

This principle may be applied to a general scheme for the organization of the spinal centers, shown in figure 4, which is simpler than that in figure 2, and yet I believe, provides for all the phenomena called for in the discussion. A great difficulty in the original scheme was to picture a pre-motor neurone with so brief a refractory phase

as to respond with separate impulses to the rapid sequence of afferent impulses reaching it almost simultaneously. If we dispense with the assumption of convergence as the basis of inhibitory frequency in the extensor center, and refer this frequency instead to the breaking up of a single impulse into a series of briefer ones in the finer branches, we may establish the conditions for inhibition on the principle just explained in the schema shown in figure 3. The rest of the scheme will operate substantially in the manner outlined in connection with figure 2, with the modification that excitation in the extensor center depends largely on the summation effect of convergence just explained (fig. 3). The release of the flexor motor neurones from inhibition by the side fibrils of the extensors in the case of a dominant flexion reflex will depend on inhibition of the extensors. The same considerations of preponderance of numbers of neurones will apply as before, in the case of crossed inhibition of flexors.

This simplified scheme presents whatever advantage there is in unification of the principles involved. It affords a possible, if not probable, cause of the various impulse frequencies, instead of merely postulating them.

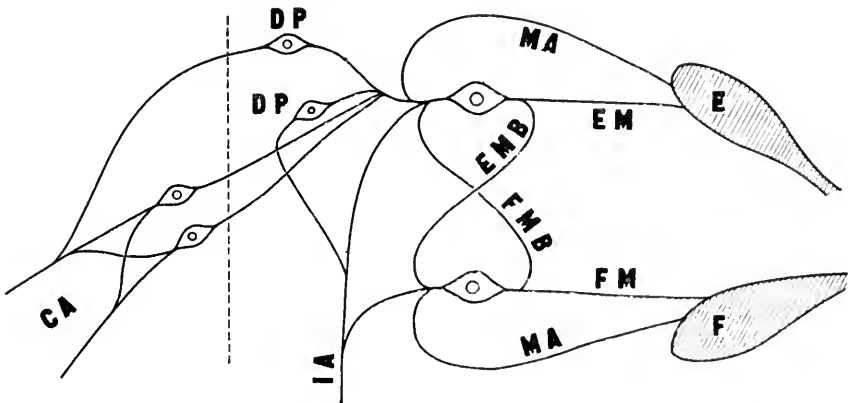


Fig. 4. Simplified scheme of spinal neurones. Lettering as in figure 2.

#### CONCLUSION

Any elaboration of a schema such as those presented is apt to be construed, in spite of earnest professions to the contrary, as an attempt to endow a provisional hypothesis with the dignity of a well-founded theory. Again I wish to emphasize that neither schema as outlined is advocated as being at all probable. They are developed merely as samples of the kind of possible arrangement whereby we may test the question whether our present knowledge of reflexes reveals anything that cannot be reconciled with Lucas' proposed interpretations.

Our view of so large a problem must necessarily remain inconclusive until our knowledge has advanced far beyond its present stage. But when we consider the enormous complexity of connections in the nervous system, and the great range of possible variation in the time relations of response in the different parts, depending on structure and dimensions, we may well believe that a basis for all the great diversity of function may lie in the single type of disturbance which seems to be a phenomenon common to nerve and muscle fibers. We should therefore refrain, at least for the present, from concluding that summation, inhibition, tonus, and all the other phenomena characteristic of the

central structures require the assumption of new and wholly unknown functional capacities in the central mechanism.

The question raised by Lucas is still unanswered. But if the time comes when it can be shown that the evolution of the dominant activities of man has depended essentially on the elaboration of a single process, the development of the branched and attenuated fiber with an unstably polarized membrane appearing in enormously varied forms, but ever the same in its basic properties, this will be a generalization of the first magnitude.

Such a generalization need not affect any philosophical position we may take as to the relation between cerebral activity, as viewed from the objective standpoint, and consciousness as known to us through our own subjective experience. It should not seek to rob conscious life of any of its subjective properties. It would merely, if substantiated, effect a simplification of the physical aspect of the activity of the nervous system.

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## AUTOLYSIS AND ATROPHY

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Atrophic changes in mass of body tissues are brought about by chemical liquefaction of the tissue proteins, catalyzed by enzymes present in the cells. In the course of this liquefaction the same products are formed which appear as the end result of hydrolytic cleavage of protein by the enzymes of the digestive tract, namely, the peptides and amino-acids. By diffusing out into the blood and lymph, these products are removed and the cell or the tissue decreases in mass and in metabolism to a proportionate degree. In certain atrophies, digestion of the tissue proteins is further facilitated by the action of phagocytes, but even in these cases phagocytosis is a secondary process, and must be preceded by the initial steps of autolysis or post-mortem changes which produce chemotactic substances attractive to the phagocyte.

The early work in autolysis has been so ably reviewed elsewhere by Levene, Wells, Dernby, etc., (1), (2), (3), (4), (5) that in this paper we may consider it only in its broadest aspects and omit the detail. This becomes all the more necessary in view of the difference in attack and technique in the earlier work, the neglect of certain factors such as the H-ion concentration, which is of fundamental importance, and the influence which point of view seems to have exerted in the collection and interpretation of data in certain instances. Those pieces of work have been selected for discussion which have contributed important steps to the advance of our knowledge of the mechanism of autolysis and atrophy.

The earliest scientific study of the phenomenon of autolysis was that of Salkowski (6). He described the process correctly as one of self-digestion of the tissue proteins and pointed out its fundamental identity with digestion in the alimentary tract. It is doubtful however whether Salkowski and his students (7), (8), (9) appreciated the importance of this phenomenon in its relation to intermediary metabolism. It appeared as an interesting post-mortem disintegration. Salkowski named the process "auto-digestion" and showed it in liver, spleen and other tissues by the production of leucine and tyrosine, which could be isolated and identified.

In 1900 Martin Jacoby (10) published his classic papers on autolysis and its relation to the atrophic disintegration of the liver in phosphorus poisoning. Later a third paper (11) appeared in which the specific action of liver protease was shown. Jacoby approached the problem from the angle of the pathologist and was therefore struck by the outstanding facts that *a*, normal liver autolyzes, producing typical end products of protein cleavage; *b*, phosphorus-poisoned livers digest more rapidly and more completely than the normal; *c*, enzymes may be liberated into the blood stream during the acute atrophy of phosphorus poisoning, which digest fibrinogen, so that the blood may not clot at all, or if it does clot, the fibrinogen speedily liquefies again; *d*, the process goes on with the animal and tissue still alive, and must therefore be of much greater import than as a post-mortem phenomenon only.

Much of the subsequent detailed work in this field was anticipated by Jacoby. He separated the enzymes from autolyzing liver by precipitation with ammonium sulphate and showed that they accelerated the decomposition of a normal liver mixture. He observed that the residue after prolonged autolysis consisted of nucleins, connective tissue debris, and a soluble albumin. The globulin fraction had entirely disappeared. The amino-acids do not accumulate in a liver with circulation intact, but if a lobe be tied off for several hours in the surviving animal they can then be found. Even the liver of the phosphorus-poisoned dog shows no amino-acid accumulation until the agonal stage is reached, when the hepatic circulation is much depressed. Although phosphorus poisoning of the liver leads to its rapid disintegration *in situ*, the addition of phosphorus to a liver hash does not alter its rate of autolysis. There appears to be a certain specificity of action of the liver enzymes, since they do not digest lung tissue. On the other hand, if proteoses and peptones are added to a liver hash, these are digested by the liver proteases. This observation evidently anticipates the discovery of erepsin in tissues. Jacoby used antiseptics in his experiments, particularly an excess of toluol.

Following Jacoby's publications, the field of autolysis at once attracted investigators, and the next two decades saw an enormous accumulation of data concerning this mechanism.

The technique used varied with different investigators. In the main it consisted in grinding tissues fine, adding water and an antiseptic, or attempting to carry on the process under aseptic conditions. An initial sample was analyzed, usually by coagulating the proteins with heat and testing the filtrate for total nitrogen, protease, peptone, amino-

acids, ammonia, etc. Or precipitating agents were used to remove protein, such as tannic acid, colloidal ferric hydroxide, trichloroacetic acid, etc. This was an improvement since the hydrolysis of the proteins during heat coagulation must always introduce some uncertainty in the interpretation of the results.

Some interesting work was done on the press-juice of tissues rather than the comminuted organ. Results obtained on such material however cannot be taken as representing the autolytic picture in the whole tissue, so that it loses some of its value. This fact is clearly seen in the results of Hedin and Rowland (12) on muscle press-juice. This material digests about as well in alkaline media as in acid, while we find that whole skeletal muscle behaves like other tissues, digesting best in acid media, and being inhibited in alkaline.

The results obtained under aseptic conditions, rather than with antiseptics present, must always remain open to the suspicion that the digests were not sterile. Even where routine cultures were found negative, it does not by any means prove that bacteria were not present, as was shown by Wolbach, Saiki and Jackson (13), (14). These authors found evidence that bacteria may be present regularly in dog liver, which require however very special culture media to grow them outside the liver tissue itself. Wherever hydrogen sulphide is noted as a product of autolysis, or where the liver mass turns dark, as described by Magnus-Levy (15), the presence of bacteria is practically certain. Thus a considerable number of papers on autolysis must be accepted with reservation on this account.

On the other hand, the antiseptic digest must be equally carefully scrutinized since antiseptics may alter the autolytic rate very materially. Boric and salicylic acids unquestionably accelerate the process (16) so that the autolysis is exaggerated far above that for the normal tissue. Apparently one of the best antiseptics thus far described is toluol, which does not alter the proteins nor destroy the enzyme, nor greatly alter the speed of the reaction in either sense, but which is sufficiently active as a germicide to inhibit bacterial growth, if the mass is kept saturated with it. Jackson (17) found that toluol decreased autolysis considerably but his control digests were admittedly infected so that the greater speed of the aseptically set up digests is to be attributed to the action of bacteria. In practice it is found that shaking a digestion bottle with toluol, present to the extent of about 10 per cent by volume, every few hours for the first twenty-four, suffices completely to destroy or inhibit bacterial growth. Such a digest will then remain for years in the thermostat without the slightest evidence of putrefactive change.

Autolysis may be measured by the amino-acids produced, or by intermediate cleavage products. The two fractions represent essentially different phases of the process. As a criterion of total autolysis, the amino-acids are undoubtedly the best. They may be titrated directly by the Sørensen method (18), or decomposed by the Van Slyke technique (19), or they may be estimated indirectly by depression of the freezing point (20) and by conductivity determinations (21). If ammonia is split off in the process, it will be titrated as amino-acid by the Sørensen method, of course, so that allowance should be made for ammonia production in strictly interpreting amino-acid titration figures. The products of primary cleavage are precipitated by tannic acid, trichloroacetic, or phosphotungstic acid from the filtrate from heat coagulation, and the total nitrogen determined. Inasmuch as primary protein cleavage liberates tyrosine-containing peptides which will react with the phenol reagent of Folin and Denis (22), this method may be used as a colorimetric estimation of initial cleavage (23).

Ammonia liberated in the course of autolysis can easily be estimated by the aeration technique, Nesslerizing the distillate. Some of the early work indicated that ammonia was split off in extraordinary amounts during autolysis, and this mistake is widely quoted in the literature. Where unusual ammonia values were obtained, putrefactive processes were probably going on (24). Later work (25) has clearly shown that deamination of the amino-acids does not take place to any extent in the *in vitro* experiments. Ammonia is produced slowly, increasing over a long period of time. It is not formed from amino-acids but from acid amides decomposable by hydrochloric acid. Dakin showed that if the total acid amide in kidney digests was determined by heating with strong hydrochloric acid, autolysis merely increased free ammonia at the expense of the acid-amide fraction. Deamination of amino-acids probably requires the active oxidative reactions of living tissue. While the deamination of purine bases occurs (26), (27), (28), the amount of ammonia derived from this source is not large.

*Results of earlier work.* The more important results of earlier work in this field, weighed critically in view of the technique employed, established the following important generalizations.

1. All tissues examined (the connective tissue group is not included) will autolyze to a greater or less extent (29). The epithelial tissues such as liver, thymus, thyroid, mammary, salivary, kidney and other gland tissues, hydrolyze most rapidly and most completely. Muscle tissue autolyzes to some extent, but much less than gland tissue. Heart muscle stands intermediate.

Vernon (30) showed that the ereptic activity of mammalian tissues had the following relations:

TISSUE	ACTIVITY (MEAN)
Kidney.....	14.3
Pancreas.....	6.4
Spleen.....	7.6
Liver.....	5.0
Cardiac muscle.....	1.59
Skeletal muscle.....	0.77
Brain.....	1.24
Gastric mucosa.....	3.9
Duodenal mucosa.....	27.7
Jejunal mucosa.....	18.2
Ileal mucosa.....	14.4
Large intestinal mucosa.....	5.8

While this table gives only the ereptic values it nevertheless shows approximately also the total comparative autolyses.

2. Tissues like the liver, kidney, thymus, etc., are found to autolyze more rapidly in the presence of quite a variety of substances. A partial list of these accelerators includes the sulphate, oxalate and chloride of iron, the chloride, sulphate, acetate and lactate of manganese, gold and platinum chlorides, aluminium sulphate and chloride, cobalt chloride and nitrate, lead acetate and nitrate (31), (32), (33), (34), (35). The following compounds of arsenic have been reported as accelerating the reaction when present in minute amounts:  $\text{As}_2\text{O}_3$ ,  $\text{Na}_2\text{HAsO}_3$ ,  $\text{K}_2\text{HAsO}_3$ ,  $\text{CaHAsO}_3$ ,  $\text{Na}_3\text{AsO}_4$ ,  $\text{K}_3\text{AsO}_4$ ,  $\text{AsCl}_3$ ,  $\text{AsBr}_3$ ,  $\text{AsI}_3$ ,  $\text{As}_2\text{S}_3$  and several others (36). In general all of the acids which do not precipitate the proteins are found to accelerate the reaction, even including such very weak acids as boric, salicylic, benzoic, carbonic acid and hydrogen sulphide (39), (40), (41), (42), (43), (44), (45). Iodine and bromine in small amounts accelerate autolysis, while chlorine has not been tried (46), (47) (48). A large number of inorganic hydrosols are reported as accelerators by Ascoli and Izar (49), (50), (51), (52), (53), (54). They include colloidal Ag, Pt, Au, Hg, Pb, Fe, Cu, Pd, Ir,  $\text{Fe}(\text{OH})_3$ ,  $\text{MnO}_2$ ,  $\text{Al}_6\text{O}_{11}\text{H}_{10}$ ,  $\text{As}_2\text{S}_3$  and  $\text{Sb}_2\text{S}_3$ . Silver nitrate and lactate, and mercury salts in very small amounts, are also found to increase autolysis (37), (38).

It is clear that the majority of the substances reported are either acids or hydrolyze in water to form acids. The other groups of accelerating substances must depend on some other factor, less obvious, for their accelerating effect.

3. Tissue autolysis may be definitely prevented or inhibited by  $\text{NaHCO}_3$ ,  $\text{NaOH}$ ,  $\text{CaCO}_3$ ,  $\text{ZnO}$ ,  $\text{MgO}$  and other basic compounds which either make the brei alkaline or keep it neutral (55), (56), (57), (58), (59), (60), (61). Other inhibitory agents are the ions of some of the heavy metals at considerable concentrations which may combine with and precipitate the proteins and also probably the enzymes; arsenious oxide in considerable amount, and formaldehyde (62). Alcohol at fairly high concentrations inhibits, but autolysis may continue slowly in surprisingly high percentages as was shown by Wells (63).

4. Some foreign proteins introduced into a liver brei are digested (64), (65), (66), (67), (68), (69). Gelatin, proteose and peptone are digested rapidly, casein somewhat less readily. While some other foreign proteins digest, the evidence is far from complete in establishing the availability of foreign proteins in general for hydrolysis in autolyzing tissue mixtures, and no very definite conclusions are warranted on the subject of specificity. Some protein preparations definitely inhibit autolysis as for example blood serum and egg albumin (70), (71), (72), (73), (74).

In brief, the speed of the autolysis and its extent are profoundly influenced by the reaction of the tissue mixture. If the tissue is made alkaline or kept neutral, there is practically no evidence of autolysis, or it is at best an exceedingly small figure. If the tissue mixture is made acid, both the rate and the extent of autolysis are increased, as measured by amino-acids produced. It is certain that most of the substances studied which do accelerate autolysis, do so by virtue of their acidity. The increase in rate and the extent bear a rough proportionality at least to the amount of acid added, till an optimum concentration is attained. This optimum is different for different acids, which indicates either that the  $\text{H}^+$ -ion influences the rate of autolysis or the negative ions have characteristic effects. At this reaction over 90 per cent of the liver proteins and those of other gland tissues appear as cleavage products. The insoluble residue is mainly connective tissue stroma. In the case of muscle the per cent of the total autolyzed is much less. The amino-acid curves of autolyzing acidified tissue are apparently identical with curves produced by adding some outside substratum, like gelatin or peptone.

Applying these generalizations to the problem of atrophy we may conclude that anything which tends to increase the acidity of a tissue *in vivo* will result in autolysis just as it does *in vitro*. Anything which maintains a tissue at its normal faintly alkaline reaction will prevent

autolysis. When a tissue is alive and in place it does not atrophy unless it is injured directly, as by trauma, pressure, poisons, etc., or unless its blood supply is interfered with. In all such cases the normal metabolism is deranged, acidity develops, and atrophy results. Normal tissue has the same H-ion concentration as the blood, or  $\text{pH} = 7.4 \pm$ . As long as this is maintained there is no evidence that autolysis can go on, either *in vitro* or *in vivo*.

*H-ion changes.* When a normal tissue is removed and examined at once it is found to be neutral or faintly alkaline to litmus. On standing it grows acid. Morse (75) studied the H-ion changes post mortem by the indicator method and found fairly rapid rise of the H-ion from about  $\text{pH} 7$  to  $6$ , which it approximates in 5 days and then remains constant. This reaction has been more exactly investigated by Dernby and also in this laboratory by the potentiometer method (76), (77). While it has not been possible to make determinations at the instant of removal, we have found soon after death a reaction of  $\text{pH} = 7 \pm$ . Acidity develops rapidly and within 24 hours the H-ion of the mixture reaches the level of  $\text{pH} = 6.5 \pm$ . For the next ten days the reaction drifts slowly back toward the neutral point as autolysis proceeds, usually reaching a level of  $\text{pH} 6.6$  to  $6.8$ . This may be referred to the gradual buffering of the acid by peptides and amino-acids, neutralization by ammonia, or to some other factor. After ten days the reaction slowly grows more acid again so that in twenty days it usually reaches a level of  $\text{pH} = 6.5 \pm$ .

The rise in the H-ion concentration is explained by the production of organic acids such as lactic incident to the dying of the tissue and post-mortem changes. While considerable work has been done to determine the amount of acids produced and their variety, this phase of the autolytic machinery is still in need of further investigation. Lactic acid has been described and isolated by a number of workers, and it has been shown that leucocytes and tissues can form it from the hexoses in small amounts (78), (79), (80), (81), (82), (83), (84), (85), (86). It appears from H-ion measurements which we have made that added glucose has little measurable effect on the H-ion changes nor does glucose appear to alter the autolytic rate. Another source of acids is the fat of the tissue, and in the liver this is often considerable. We have found that such fats as cottonseed oil, cod-liver oil, olive oil, etc., lead to an increase of the H-ion concentration of an autolyzing liver brei to which they are added, and as they hydrolyze the fatty acids compete with the tissue proteins for the basic ions. The net result is

that digestible fats in a liver brei increase its autolysis. Post-mortem development of carbon dioxide plays a part in the rise of acidity, but so small as to be almost negligible. It does however largely increase the rate and extent of autolysis if added in sufficient amount.

The significance of the slow drift toward neutrality during the first ten days followed by an equally slow drift in the acid direction during subsequent periods is not understood. It is probably of no importance in actual atrophy where diffusible material is carried away. The initial acid production is in any event the determining factor in the extent of autolysis, and in those tissues where much acid develops autolysis is correspondingly large.

Two factors must be distinguished in the increase of autolysis by acid. One is the *total acid present*, and the other is the *H-ion concentration*. Both effect the reaction but in different ways. The enzyme effecting the primary cleavage of the tissue proteins ("Beta protease" of Hedin (87), the "endotrypsin" of Hahn (88), and the "pepsin" of Dernby (89)), is active only within certain H-ion limits and it has an optimum environment at which it catalyzes hydrolysis most rapidly. In the case of liver (pig) we have found the optimum reaction about  $\text{pH} = 4.5 \pm$ . This enzyme is probably quite inactive at  $\text{pH} = 2.6$ . These figures agree fairly well also with those reported by Dernby. As the H-ion concentration rises therefore, after removal of the tissue, the primary protease becomes more active, up to the optimum. At the same time the amount of acid developed in or added to a digest will determine the amount of amino-acids ultimately produced by determining the mass of substratum, and this will exert a mass effect in increasing the rate of amino-acid production up to the point where the enzyme is saturated with substratum. As acid is added to a digestive mixture, both factors are operative and it is difficult to differentiate the effect of one from the other. It can be done however experimentally by introducing equivalent amounts of acids of different dissociation constants. Thus in two digests to which the equivalent amounts of hydrochloric and acetic acids are added, we find much more rapid amino-acid production going on in the hydrochloric acid mixture. It has a higher H-ion level than the one with acetic acid present. In the course of ten days however the two digests have approximated equilibrium at the same level. The higher H-ion given by the mineral acid produces more rapid catalysis of the substratum available, but this mass is about the same in both digests, and this determines the final amino-acid production when equilibrium is attained.

*In vivo* the acids normal to metabolism are all such as to give relatively low H-ion concentrations. They are carbonic, lactic, butyric, with some sulphuric and phosphoric from the combustion of proteins and lipoids, and possibly other weak organic acids. Such acids would suffice to increase the mass of substratum, but the rate of its hydrolysis would not be very rapid. Under certain pathological intoxications mineral acids are produced in considerable amount, and in these instances autolysis is found to be both rapid and extensive.

The addition of acid quite evidently increases the mass of substratum for autolysis considered as a whole. This substratum we believe to be the acid-protein produced by the addition of acid to the base-protein salts of the normal tissue. This explanation seems all the more plausible when curves of autolyzing liver to which acid or gelatin are added, are compared. In both cases rate and amount of amino-acids produced increases in such a similar way that we are convinced the effect is actually the same—the effect of increased mass of substratum.

*The effect of colloids.* There is one series of observations that cannot be covered by this explanation. The extensive contributions of Ascoli, Izar and their co-workers (90) demand some special consideration, both because of the very large amount of data presented and because of its possible effect on the understanding of the subject as a whole. The first of a series of papers on the effect of colloidal sols on the autolytic reaction appeared in 1906. In this paper the metallic colloids of silver, gold and platinum were reported as increasing autolysis in a striking fashion. The three behaved alike qualitatively and almost quantitatively. The authors conclude that these metallic colloids catalyze the autolytic hydrolysis just as they catalyze the decomposition of hydrogen peroxide. These results seem to offer a reasonable explanation for the increased nitrogen catabolism resulting from injections of such metallic hydrosols (92), (93), (94), and silver salts (91).

In subsequent papers the same observation is extended to include the list of colloids previously enumerated. The sols were stabilized with an amount of gelatin too small to produce a measurable effect on the amino-acid figures through its own digestion. They all increased autolysis from 50 to 100 per cent or even more. The inspiration of Bredig's classical work on the *Anorganische Fermente* (95) is unmistakable throughout the series, even to the confirmation of the "toxic" effect on these catalysts of such substances as phosphoric acid, mercuric cyanide, etc. It is difficult to escape the impression that much of this work was conducted with a definite mental bias which inevitably influenced the

collection of data. This impression is intensified by analysis of the data in the papers. For example, the addition of hydrochloric and phosphoric acid in amounts which regularly increase autolysis in the hands of other investigators failed to accelerate the process. This is entirely contrary to all the evidence of autolysis before or since. Furthermore we have repeated many of the typical experiments described, and are unable to confirm the results (96). It is true that certain colloidal preparations increase autolysis. In all cases which we have studied, the effect is due to acid, however. Commercial colloidal ferric hydroxide contains much ferric chloride which hydrolyzes to hydrochloric acid and produces a typical acid increase of autolysis. When however this hydrosol is dialyzed for long periods until no further chloride can be removed, it is completely inert.  $\text{As}_2\text{S}_3$  and  $\text{Sb}_2\text{S}_3$  both accelerate autolysis, not because they are colloidal, however, but because in water they slowly decompose and give up  $\text{H}_2\text{S}$ , which accelerates autolysis. Sols of gold, platinum and silver have been prepared by the Bredig method of arcing under pure water, and by reducing reactions. But in no case have we observed the slightest effect on autolysis if all traces of acids are removed. We have reluctantly come to the conclusion that much of this voluminous contribution to the literature of autolysis is unreliable and so largely incorrect that it must be disregarded until it has been reinvestigated *in toto*. In later papers Izar and Truffi (97), (98) report that minute additions of silver and mercury salts will greatly increase autolysis. These results we have also repeated but are unable to confirm the observation.

*The autolytic enzymes.* Jacoby described an enzyme of the liver as erepsin-like, since it would digest proteoses and peptones of autolyzing lung, but would not attack the lung tissue proper (99). Jacoby isolated the proteolytic enzymes of liver also by precipitating with ammonium sulphate. He believed them to be quite specific.

Hedin (100) separated two enzymes from the spleen—"alpha protease" which digests in alkaline media, and "beta protease," digesting in acid media. Buchner and Hahn described an "endotrypsin" which they obtained in the autolysis of yeast, resembling trypsin in its ability to carry protein cleavage to amino-acids, but differing from it in acting best in an acid medium (101).

Of the recent papers bearing on this question, the two by Karl Dernby (102) undoubtedly are of first importance. For the first time the control of the H-ion concentration was perfected in this investigation. In the first paper Dernby presents the data obtained in a study of

the autolyzing enzymes of yeast. It confirms the earlier suggestions of Hahn, Hedin and others, that there is present in yeast an enzyme which hydrolyzes the native protein to the peptone stage, acting best at about pH 4.5, a trypsin-like enzyme producing amino-acids from casein, gelatin and peptides and most active in a reaction of 7.0, and an ereptic-like enzyme, producing amino-acids from the peptides at a pH of 7.8, and having the specific property of the erepsin of the intestinal mucosa of splitting glycyl-glycine

The optimal reaction for total autolysis is about pH 6.0, or slightly on the acid side of neutrality. At this point, as Dernby shows, the simultaneous action of the three enzymes is possible. At a higher acidity peptic hydrolysis proceeds, but the tryptic and ereptic action is inhibited, while at about the neutral point the peptic action is arrested and the tryptic and ereptic cease for lack of substratum as soon as the preformed peptides are hydrolyzed. The enzymes are studied in great detail, the effect of various added ions determined, the kinetics of the ereptic activity evaluated, and a mass of accurate data of the greatest value secured.

In Dernby's second paper a similar study of tissue autolysis is made, and the results are interpreted in a similar way. There seems however valid reason for questioning the interpretation. In effect the article describes the autolytic process as catalyzed by the three typical digestive enzymes pepsin, trypsin and erepsin. Two of these names carry a very definite implication in regard to their properties. They have applied for decades to the enzymes of the digestive tract, their optimal H-ion has been exactly measured, their products determined and the substrata on which they act. It is quite clear from Dernby's own results, and from our own (103), that the resemblances are not very close except in the case of erepsin and it is believed that the use of these historic names in this connection can only lead to confusion of ideas. It is not our intention to quibble over names, but in this case the application of the terms pepsin and trypsin, whose definition is clear from the research of decades, to enzymes which do not closely resemble in several important respects the originals, is questionable.

The enzyme in liver which Dernby calls "pepsin" resembles gastric pepsin in facilitating the primary cleavage of the native tissue proteins made sufficiently acid. It probably does not produce free amino-acids in this cleavage any more rapidly than does gastric pepsin. On the other hand, the liver enzyme has an optimal H-ion concentration of pH 4.5 while pepsin acts best at pH 1.5  $\pm$ . The liver enzyme is com-

pletely destroyed before this latter H-ion level is reached. This constitutes a very obvious and probably fundamental difference. It can hardly be associated with the precipitation of some protein fraction of liver tissue and the consequent removal of the enzyme by adsorption in this accidental way, since pepsin added to a liver brei is not so destroyed but acts at its highest speed. Pepsin is further characterized by the very wide variety of native proteins which it will hydrolyze under proper acidity. From some of our own unpublished experiments the primary protease of the tissues is more specific. Further work however is necessary before definite conclusions can be drawn in this particular. The apparent "activation" of this enzyme by acids may be due to the higher H-ion level, or it may be referable to the development of substratum in the tissue at an H-ion concentration above pH 7.0 or it may be and probably is due to both. It is clear at least that the base-protein salts which exist in the living tissue are not attacked by this enzyme, and the tissue as a whole therefore does not undergo autolysis. When acid is added, however, this is converted into free protein or acid-protein. At the same time the H-ion rises and the activity of the protease increases. Digestion of the acid-proteins proceeds, with primary cleavage producing peptides of a character which may then be further digested by the second enzyme complex, or erepsin. Autolysis as a whole is thus found to be roughly proportional to the acid added, and to the H-ion level also.

If the initial cleavage depended solely on the increasing *activity* of the primary protease in acid solutions, then it would appear that at a slight increase of H-ion level above pH 7 digestion of the tissues should be slow, but complete in time. This however is not the case. With each increment of acid, the total autolysis increases by a similar increment. The reaction goes rapidly at first and later reaches equilibrium. Equilibrium appears to be very definitely determined by the amount of substratum available, and this amount appears determined by the increment of acid. More or less of the tissue is digested, therefore, as more or less of it becomes dissociated by acids stronger than the proteins themselves.

The argument for the application of the name "trypsin" to one of the proteases found in liver or kidney is still less justifiable. Trypsin acts best between pH 7 and 8. It digests most of the native proteins and produces amino-acids as the end products. Complete cleavage proceeds so rapidly that there is little accumulation of proteose or peptone fractions in a typical tryptic digest. In the liver and kidney we find

no enzyme which answers this description. The native tissue proteins appear undigested at pH 7 after months, and the amino-acid production is negligible. This is not due to any resistance of these tissue proteins to tryptic digestion at this H-ion level, for if trypsin be added to such a liver brei digestion goes on rapidly and completely, with the exception of the connective tissue residue. There seems to be no evidence therefore of a tryptic enzyme in the tissues which corresponds to the trypsin of the pancreatic juice and gland.

Erepsin, or the ereptases, on the other hand are widely distributed (104), (105), (106), (107). They are characterized by their inability to cleave the native proteins, casein excepted, but act upon the polypeptides, converting them into amino-acids. According to Abderhalden (108), there is an optimum H-ion concentration for the cleavage of each peptide, but the average is in the neighborhood of pH 7.8. Dernby has shown by the splitting of glycyl-glycine that a true erepsin exists in yeast and in such animal tissues as he examined. While it differs from the intestinal enzyme in its sensitiveness to various foreign ions, there seems to be no good reason for classifying it in a separate category.

While the tissue erepsin acts best at about pH 7.8, it is still active in as acid a medium as pH 3—. This is shown by adding a little pepsin to a liver digest made up to this H-ion level. Autolysis alone is completely inhibited at this acidity. Pepsin however effects the initial cleavage, and the erepsin present carries on the hydrolysis to the amino-acids rapidly. If the H-ion be raised to pH 1.2— the liver ereptase is completely inhibited, although the pepsin is very active. The amino-acids appear only at the very slow rate characteristic of the action of pepsin alone. In the living tissue the activity of erepsin is evidently conditioned by the production of primary cleavage products. These in turn are conditioned by the development of acidity within the cell. So long as the buffer mechanism within the cell maintains its normal reaction autolysis is completely in abeyance.

To summarize, we find that the enzyme mechanism of autolysis comprises at least two groups of proteolytic enzymes. One acts only in acid media and converts the acid tissue proteins into primary cleavage products. It is active between pH 7— and 2.6—, and its optimum is about 4.5. The second attacks only the primary cleavage products of the proteins, producing the amino-acids. It is active between pH 8— and 3, and seems to be identical with the erepsin of the intestinal tract.

*The mechanism of atrophy.* From the data presented above we may form what is probably a fairly accurate hypothesis for relating autolysis

to the various normal and pathological atrophies. The normal tissue cell is maintained at the reaction of the blood, or pH 7.4. So long as its metabolic processes remain in equilibrium with its blood and lymph supply there is no accumulation of carbon dioxide, sulphuric acid, phosphoric acid or acids of intermediary metabolism. Neutralization of these acids, further oxidation of some of them, diffusion out of acid ions and diffusion in of basic ions goes on at such rates that the resultant reaction remains a constant.

Under these conditions the cell proteins are in the form of base-protein salts (109). The primary protease is inactive, the ereptase active but inoperative, and the amino-acids present in the cell are in equilibrium with the proteins and in diffusion equilibrium with the amino-acids of the blood. Any prolonged increase of metabolism within the cell without compensatory increase of the circulation about it would lead to an acidotic shift within the cell. Such shift, resulting from excessive acid production, leads to a rise in the H-ion and a change from base- to acid-protein. Primary cleavage results and the products are further disintegrated by the ereptase. The excess amino-acids diffuse out into the blood and lymph until an equilibrium is again attained. The net result of these steps is a cell of decreased mass and presumably of decreased metabolism, and the decrease continues until accurate adjustment between the cell and its blood supply is reestablished. Any increase in acid production in cell or tissue beyond the capacity of the buffer mechanism to immediately dispose of must automatically tend to atrophic changes of mass.

We may apply this conception to a number of typical atrophies representing both normal and pathological conditions. The interpretations here offered are not all of them definitely proven. They represent however the field in which many of the future problems of autolysis must be worked out, and are therefore presented as tentative interpretations subject to change with new data.

1. The liver is particularly liable to intoxications and atrophic changes. Phosphorus poisoning leads to an acute atrophy in the course of which the entire liver is apparently destroyed and autolyzed. Phosphorus accumulates in the liver probably in the lipid phase in which it is freely soluble. Like all strong reducing agents it produces asphyxial conditions in the cell which lead to acid development. In addition phosphoric acid is probably produced in the oxidation of the phosphorus. While phosphorus added to a stoppered liver digest does not increase autolysis, as Jacoby showed, it can be made to do so by passing a stream

of air through the mixture. In any event the liver cells become markedly acidotic, die, and autolyze rapidly and completely.

2. In phosgene poisoning the alveolar lining of the lungs swells, disintegrates, sloughs off, and in general gives every indication of rapid and complete autolysis. Phosgene is a gas which is soluble in both lipoids and water. In the latter it is rapidly hydrolyzed thus:— $\text{COCl}_2 + \text{H}_2\text{O} = 2 \text{HCl} + \text{CO}_2$ . The gas penetrates the alveolar cells almost instantly and is as rapidly converted into hydrochloric acid. The cells die and begin autolyzing at exceptional speed on account of the high acidity developed within them. Chlorpicrin and other asphyxiating war gases owe their toxicity to this mechanism.

3. "Mustard gas" is perhaps the most striking example of a poison depending for its action on penetration followed by acid production. Dichlorethylsulphide is soluble in lipoids and fat solvents; it volatilizes slowly, is only slightly soluble in water, and hydrolyzes as follows:  $(\text{C}_2\text{H}_4\text{Cl})_2\text{S} + 2\text{H}_2\text{O} = 2 \text{HCl} + (\text{C}_2\text{H}_4\text{OH})_2\text{S}$ . Marshall and his co-workers (110), (111), (112) have shown that "mustard" penetrates the skin by virtue of its organo-solubilities. Penetration is very rapid to the active dermal cells where the material concentrates in both the water and lipid phases. Hydrolysis goes on slowly so that some hours after contact the developing hydrochloric acid starts the autolytic mechanism and eventually kills the cell. The rapid blistering and necrosis after the process first becomes evident is an expression of the speed with which the autolysis goes on under these conditions of excessive development of a strong acid within the cells themselves. In severe burns the whole area may digest and slough off, producing a lesion of marked chronicity.

The hydrolysis of "mustard" within the cell was definitely demonstrated by Lillie, Clowes and Chambers (113), who found that starfish eggs in water containing freshly dissolved gas absorbed some of it, and after a latent period during which development was normal, underwent necrotic changes and died. The products of "mustard" hydrolysis however had no effect on the developing eggs since they did not penetrate them. If the material was injected by micro-pipette into the cell there followed a similar latent period and then rapid degeneration. An equivalent amount of hydrochloric acid injected produced the necrotic changes of a similar grade at once.

In a further study of derivatives of "mustard" Marshall and Williams (114) found that those derivatives which had solubilities similar to "mustard," and on hydrolysis produced acids, were also the derivatives which were effective in producing skin burns.

4. The liver is subject to injury by toxins of biological origin (115), (116), (117), (118). Thus the injection of hemagglutinins, tetanus and diphtheria toxins, streptococci and their decomposition products, has been found to injure the liver and lead to necrotic changes suggestive of some clinical conditions such as the liver in eclampsia or acute yellow atrophy. Jackson and Pearce studied the autolytic mechanism induced by injections of hemagglutinins. In certain cases the animals died early with the liver intensely congested and the capillaries plugged with thrombi of fused red cells. The animals which survived the immediate effects of the injection showed either a diffuse degeneration of the liver, or an organ dotted with focal necroses. The nitrogen output in the latter type was slightly elevated but normal in its ammonia-urea ratio. In the diffuse degenerating livers the ammonia-urea ratio was abnormally high. The authors interpret this as indicating that the hepatic function was obliterated in these animals while in those with focal necroses some cells were destroyed but enough normal tissue was left to carry on the hepatic functions. It is probable that there was a general acidotic effect in the diffuse degenerating cases which would also give a high ammonia-urea ratio. The authors believe there is evidence of protein storage in the early stages of intoxication, but in view of the intense congestion of the liver described it seems more likely that the increased protein represents the excess of blood (119). However this may be, the process as a whole illustrates the intoxication of the cells with altered metabolism, together with a reduced blood supply, tending toward asphyxial conditions, injury or death and consequent atrophic changes by the autolytic mechanism.

5. Acute yellow atrophy is a striking example of an idiopathic injury to the liver cells resulting in almost complete destruction and digestion of the liver tissue. In a case reported by Wells (120), the liver had shrunk to half the normal size before the death of the individual. In this case the parenchyma had autolyzed away while the stroma remained intact or perhaps proliferated to some extent. The liver was consequently much richer in gelatin-forming tissue than the normal. Connective tissues as a class do not autolyze and are rather resistant to digestion by enzymes from the leucocytes. In our own studies we have set up digests of mature cartilage and white fibrous tissue which showed no measureable increase of amino-acids after weeks in the thermostat.

6. Chloroform causes a more or less profound atrophy of the liver depending upon the length of exposure (121), (122), (123), (124), (125), (126). From non-fatal doses the organ recovers completely. Accord-

ing to Graham, the necrosis is caused by the break up of chloroform in the liver cells through phosgene to hydrochloric acid, and this would easily account for the autolytic changes which are found under way in such a liver. Graham has also shown that other halogen compounds similar to chloroform produce similar liver lesions and in proportion to the hydrochloric acid produced. Other chlor-compounds, like chloral, do not break up with the liberation of the halogen acid and these do not affect the liver. Iodoform and bromoform both cause liver degeneration, and the excretion of iodides and bromides in the urine makes it clear that the halogen acids are produced somewhere in the body. The liver lesions make it seem fairly certain that this decomposition takes place in that organ.

7. Atrophy of the mammary gland after lactation is a typical physiological example of autolysis resulting from diminution of the blood supply. Leaving the gland partly distended with milk hastens the atrophy through pressure, which cuts down the blood supply still more.

After parturition the mammary glands in some women produce an excessive secretion of milk, become tense with pressure and often painful. This great initial pressure regularly leads to a diminution through atrophy so that within a few days the supply of milk diminishes automatically. It is common practice in such cases to anticipate the excessive secretion by bandaging the breasts soon after delivery and thus initiating the atrophic changes and avoiding the over-production. It is also the practice to bind the breasts and leave them but partly emptied during the weaning period. In both these cases autolysis is initiated by pressure stasis and asphyxial cell-acidosis resulting from it.

8. Uranium salts produce nephritis with atrophic changes in the kidney cells. MacNider (127), (128) found that the administration of bicarbonate in considerable amount decreased the extent and severity of the lesions. It seems plausible that the excess alkali helped prevent the cell acidosis and so lessened the autolytic digestion.

9. Following parturition the uterus contracts, squeezes out the blood and lymph, and in the course of a few days or weeks atrophies to its resting size. The process is commonly hastened by administering ergot or some other drug inducing contraction in smooth muscle. The sustained contractions of this organ may be assumed to cut down blood supply. Carbon dioxide will be produced in increased amount, while it and the organic acids will be less effectively removed or neutralized. An organ-acidosis results which initiates and promotes autolysis, until the organ is again in equilibrium with its blood and lymph supply.

10. Contractions of the uterus are set up at term in some way not fully understood. There may be a number of factors which are operative in bringing on the expulsive contractions, the most probable are that they are started by some chemical hormone. It seems not improbable, at least, that autolysis may be an important factor. It is well known that protein cleavage products cause uterine muscle to contract (129), (130), (131), (132), (133). A certain threshold concentration is necessary before the organ responds. Any considerable separation of the placenta in late pregnancy must alter the blood supply to the separated area. If this separation is large enough the protein cleavage products produced in its autolysis would probably be sufficient to induce contractions. The settling of the fetus as term is approached, its more and more vigorous muscular movements, are both calculated to increase the strain on the adherent placenta. If a small separation only occurs it would perhaps fail to produce cleavage products in sufficient concentration to start active contractions. It might however account for the frequent preliminary contractions so often noted as term is approached. It is well known that a fall or a severe jar or even the mild shaking incident to an automobile ride is sufficient to start the process in many cases, and it is noteworthy that contractions do not begin at once, but after a latent period of some hours. This would correspond very well with the time required after such a separation to induce asphyxial development of acidity in the placenta and the production of the early cleavage products. It has long been known that the placenta contains proteolytic enzymes similar in character to those found in other tissues (134), and we have found that it behaves to acids and alkalies exactly like other tissues. At the optimum acidity the placenta will autolyze 400 per cent faster and farther than the control sample.

11. Voluntary muscle autolyzes slowly and incompletely. It is however influenced by the tissue reaction in the same sense as the more actively autolyzing gland structures (135), (136). An inactivated muscle gets less blood than an actively contracting one. Thus the arm hanging in a sling for some weeks shows considerable muscle atrophy. With this decreased mass goes a very large part of the contractile power of the muscle. When the muscle is again allowed to contract it gradually hypertrophies again to its normal mass and strength. *In vitro* experiments show that muscle autolyzes only about a quarter of its proteins under the most favorable conditions of reaction. The rest of the tissue is not hydrolyzed. It consists of connective tissues and muscle structural proteins which are not rendered available for auto-

lytic cleavage even by acidity. It would appear that the protein fractions which are available material for the enzyme cleavage are particularly concerned with contraction, since removal of all or part of this fraction by atrophy *in vivo* removes at the same time practically all of the contractile power.

12. The atrophy of the tadpole's tail during the metamorphosis is an interesting example of the *complete* autolysis of a muscular organ. Gudernatsch (137) first showed that this atrophic phenomenon may be markedly accelerated by feeding thyroid material to the tadpole, and this has been confirmed by Morse (138), West (139) and others. It has been pointed out that the phagocytes play an important rôle in this process although Morse found no excessive number present in the tissues examined by him.

The explanation of the process is this: With the approach of the larva to maturity the bony structure known as the urostyle impinges more and more on the blood supply to the tail. Atrophic changes result. Eventually cells die and the phagocytes invade the tissue adding their less specific enzymes to those of the tissue itself. As the blood supply diminishes slowly there is opportunity and time for the complete removal of the cleavage products as they are formed little by little throughout the tail. As is to be expected, there is no gross evidence of increased autolysis on testing the tissue. Even in the fulminating autolysis of the liver in acute phosphorus poisoning Jacoby found no accumulation of amino-acids until the circulation was lessened. Nor would it be expected that the tails would show an increased rate of autolysis *in vitro*. The asphyxial changes affect only a few outpost cells at a time, so that at any one moment there would be no gross increase of acid or of cleavage products in the tail as a whole.

The significance of the results of Gudernatsch lies in the increased rate of developmental changes resulting from thyroid, not in any specific effect which it, or other iodine compounds have upon autolysis (140), (141), (142), (143), (144), (145), (146). With rapid growth of the urostyle under thyroid feeding, the train of results depending on these changes must also go on at an accelerated rate.

13. Some epithelial tissues are particularly sensitive to x-ray radiation or exposure to radium. Such cells are injured in some way by the radiation, their metabolism is interfered with and they suffer atrophic changes. If rayed sufficiently they are destroyed. In the recent studies of Warren and Whipple (147) the great sensitiveness of the epithelium of the small intestine is described. The cells in the crypts

appear more sensitive to the rays than at the tips of the villi. The microscopic picture of this tissue shows such very rapid disintegration of these cells as compared with the unrayed, that we should be inclined to suggest that autolysis was being reinforced by the enzymes of the digestive tract, trypsin and erepsin. If the cells are killed by the rays there is good reason to suppose their proteins will fix these proteolytic enzymes at once, while the protoplasm of the living cells does not have this property. Thus the dead cells carry with them to the digestion chamber enzymes in addition to the intracellular autolytic proteases, while the cells which were still living at the time the tissue was removed do not.

14. The brain is considered rather resistant to autolysis (148). This resistance is due however to its exceptional blood supply, its mechanism for increasing ventilation and blood flow to offset a developing acidotic shift within itself, and to the relatively small protein content of the brain. Of the proteins there too only a fraction becomes available in autolysis, the rest are structural and are not digested even under the best conditions. The brain behaves like other organs in relation to acidity and alkalinity (149), (150), and any asphyxial condition localized in some part of it through pressure, thrombosis, trauma, etc., results in digestion.

15. The softening of overgrowths and neoplasms is an example of autolysis induced by alterations in the blood supply. This may be caused by the contraction of freshly laid down fibrous tissue, so that capillary tufts are pinched off and their dependent tissues autolyzed.

16. In general acidosis and fevers the loss in tissue mass is correlated probably with acidotic changes within the tissue cells, leading to their partial autolysis.

17. The wastage in anemias seems also to be related to the decreased oxygen supplied the tissues with partial asphyxia and autolysis toward a new equilibrium between the mass and metabolism of the tissues of the body and their supporting blood and oxygen supply.

18. In starvation we have a striking example of slow autolysis by which a very large portion of the proteins of the body is digested and thus mobilized for the use of the more essential organs. It is evident that the tissues least subject to autolysis in starvation are those whose blood supply is most assured, the brain and heart (152). This slow autolysis is probably related to blood supply and tissue acidosis as in the other cases mentioned. It is certainly not a simple activation phenomenon as described by Schryver (151), although activation by

increased H-ion probably plays a part. Generalized acidosis is a prominent and persistent feature in starvation and it is fair to assume that it is expressive of conditions within the tissue cells. With loss of muscle proteins muscular weakness slowly increases, the individual tends to become more and more quiet. Decreased metabolism, decreased blood supply, lowered blood and pulse pressures are factors which must automatically lead to conditions favorable to autolysis (153). Thus the essential structures, like brain and heart, survive at the expense of those less perfectly supplied with blood. The skeleton persists also with very little loss because, along with the other connective tissue, it does not autolyze.

19. The very slow generalized atrophies of advancing age are probably also related immediately to a blood supply growing gradually less efficient, and leading to a concomitant decrease in the mass of tissues maintained in equilibrium with it.

These are but a few of the more obvious examples of physiological and pathological processes in which autolysis plays a rôle. Every loss of tissue mass which is not merely due to loss of water, involves this mechanism. It is safe to assume that every increase of mass, as in hypertrophy and growth, involves the same mechanism operating in the reverse direction. The factors involved in growth and hypertrophy are so much more complicated than in hydrolysis of a tissue that we know little of it experimentally. It is obvious that it requires an abundant blood supply, and a reaction which insures the stability of the base-protein complexes. The interesting observation of Menten's that in cases of carcinoma the pH of the blood is about 7.8 is suggestive (154). So too are the results obtained by Fischer in growing fibroblasts in culture media (155). The optimum reaction for growth was between 7.4 and 7.8 and the cells were more resistant to abnormally high alkalinity than to acidity. Moore, Alexander, Kelly and Roaf (156) found that fertilized sea urchin eggs are never stimulated to growth by increasing the acidity of the surrounding medium, but that they are stimulated by slight extra alkalinity, and may develop atypical mitotic figures which are similar to those seen in malignant tumors.

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## THE ORIGIN AND SIGNIFICANCE OF THE CONSTITUENTS OF THE BILE

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In attempting a review of the many constituents of the bile it is obviously impossible for the writer to include all the contributions in this field. If some important papers have received too little attention the writer hastens to apologize. It is always difficult for a reviewer to evaluate with judicial fairness the experimental data of other investigators, especially if he has been concerned with experiments which are not in accord with those under review. No review concerned with bile constituents would be complete without reference to Stadelmann's monograph, *Der Icterus* (51). It would be well if all investigators in this field were to read this book from cover to cover, for the procedure might conserve to the research journals much valuable space. Stadelmann's reviews and his experiments are models which must excite admiration in all students of this subject. It may perhaps be convenient to review each important constituent of the bile before noting certain facts concerning the whole bile.

**BILE PIGMENTS** The bile pigments may or may not be the most important constituents of the normal bile but certain it is that they have received the largest amount of attention, clinical and experimental. Bile pigments appear in demonstrable amounts in the bile canaliculi of the normal or abnormal hepatic epithelial cell. It is therefore a bit too easy to think of the hepatic cell as the only essential factor in the elaboration of bile pigment. But it is well for physician, teacher, student and investigator to keep clearly in mind that *other body cells have the capacity rapidly to change hemoglobin to bile pigment*.

Whipple and Hooper (57) showed that this transformation could be brought about within 2 hours in the blood stream of the head and thorax with complete liver exclusion. The same workers (18) showed that hemoglobin can be transformed into bile pigments within the serous cavities during a period of 12 hours or longer. McNee (31) and Van den Bergh and Snapper (54) have confirmed a part of this work. It is highly probable that the vascular endothelium and serous

mesothelium are concerned in these reactions. We wish to emphasize the point that *these reactions* in our opinion are *not physiological curiosities* but concern the normal bile pigment production. It seems probable to the writer that the Kupffer cells are concerned in the normal production of bile pigments and it is not a mere accident that these cells make up a considerable part of the gross liver tissue in the most intimate association with the hepatic parenchyma cells. It would seem possible that the formation of bile pigments might be a *function of both the liver endothelium* (Kupffer cells) and the *hepatic epithelium*, the preponderance of activity being determined by factors not understood at present. It is suggested that the Kupffer cells may be concerned with production of bile pigments from hemoglobin present in the blood stream while the hepatic epithelium may be especially concerned with the manufacture of bile pigments from other substances which (pigment complex fig. C) may be derived from the food or body cell protoplasm.

Our conception of the relations of bile pigment to other body pigments is illustrated in a crude way in figure C. Many important factors concerning the interrelation of hemoglobin, bile pigment and other body pigments have been reviewed by Whipple (56), and we must refer to that paper for much of the detailed discussion of pigment metabolism. Those interested in the chemical structure of bile pigments are referred to papers by Küster (22), (23), (24).

It has been claimed by some investigators (Brugsch, Yoshimoto, Kawashima (3)) that hemoglobin introduced into the blood stream will be quantitatively excreted as bilirubin in the bile. Whipple and Hooper (61) have been able to show that in their bile fistula dogs no such quantitative relationship holds for hemoglobin and bile pigment. It seems very probable that much of the hemoglobin set free in the blood stream may be used in the body economy for a variety of purposes—among others, the construction of hemoglobin for new red cells. For example, it is sufficiently well established that intravenous injections of hemoglobin or the destruction of red cells in the body will aid in the recovery from simple anemia with consequent upbuilding of new hemoglobin (20), (21). It is very probable, however, that the hemoglobin in the blood stream is not used directly but only after being broken down to the unit structural factors—whatever these may be. It may be difficult or almost impossible to prove beyond a doubt that hemoglobin may not be quantitatively eliminated as bile pigments in the bile but it is relatively easy to show (17) that hemoglobin

introduced intravenously, intraperitoneally or intramuscularly will aid materially in the recovery to normal from a simple anemia. If the introduced hemoglobin may be used to form new hemoglobin it is scarcely permissible to state that it is quantitatively eliminated as bile pigment. This point is illustrated graphically by double arrows between the *pigment complex* and *hemoglobin* in figure C.

It is well established (58) that the bile pigment elimination in bile fistula dogs can be increased by a change in diet—for example, a sudden change from a meat to a carbohydrate diet may increase the bile

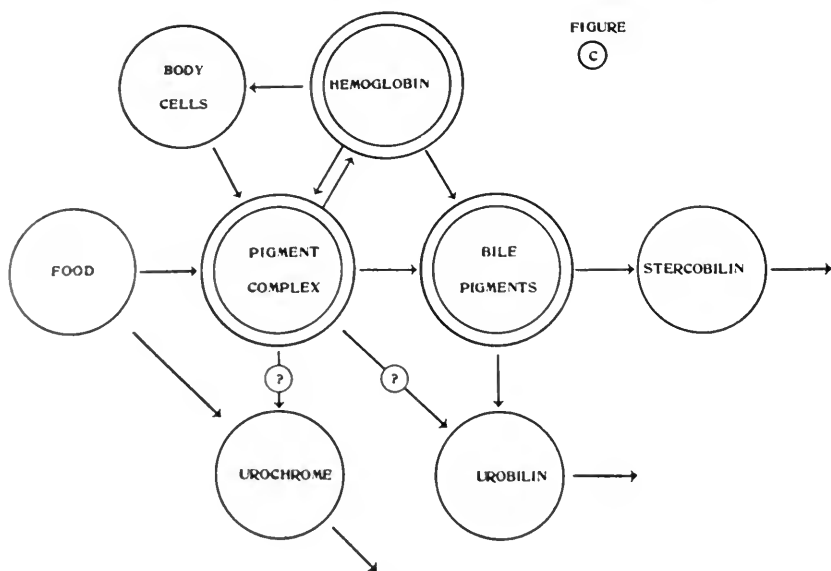


FIG. C

pigment elimination more than 50 per cent. This can be repeated time after time and it seems at least improbable that this reaction is dependent upon blood destruction. We may wish to explain this reaction in part as follows: The meat diet is normal for the dog and the increase in bile pigment excretion due to carbohydrate excess may represent an abnormal or alternative reaction—a deviation of pigment elements and construction into bile pigment for elimination. It is possible that some of these pigment elements concerned in this reaction might be available under favorable conditions (anemia) for hemoglobin construction or under usual conditions (meat diet) for elimination

elsewhere than in the bile. Urochrome is a possible end product of pigment elements and deserves much more study in normal and abnormal conditions.

The term "pigment complex" is used in figure C to indicate a group of substances which are essential parts of mature body pigments. It is obvious that certain food factors contribute to this "pigment complex," as food is directly concerned in the production of new hemoglobin and the formation of bile pigments and urochrome. It is equally clear that the body protein and cells contribute to this "pigment complex" as all body pigments are produced in measurable amounts during fasting periods. We believe the evidence is sufficient to show that hemoglobin as it disintegrates in the body also contributes to the "pigment complex" and so influences in a measure the new formation of hemoglobin. It is probable that only a small amount of the destroyed hemoglobin is conserved in this fashion. The pyrrol nucleus seems to be one of the factors which must be concerned in this "pigment complex" and it is probable that all facts related to pyrrol metabolism will have a direct relation to the complicated body pigment metabolism. The above points are illustrated graphically in figure C.

Bile pigments in the bile fistula animal are not increased by the feeding of fresh bile pigments or of fresh or cooked blood or of digestion products obtained from blood (59). This might be assumed to be from lack of absorption. There is no evidence that bile pigment or stercobilin are absorbed from the intestine. However, it has been shown that the feeding of hemoglobin will influence the curve of new hemoglobin construction after anemia (17). This indicates an absorption of substances which are concerned with "pigment complex" but it is clear that these same factors do not influence the output of bile pigments in the bile fistula dogs—at least under the conditions of our experiments. These experiments are much against the interesting suggestion of Wilbur and Addis (64) that there may be a conservation of bile pigment factors which are absorbed from the intestine and reconstructed into hemoglobin. Perhaps the strongest argument against the absorption of stercobilin and its utilization in body pigment construction is the fact that bile fistula dogs under observation continuously for 2 years or longer show no evidence of pigment lack, no anemia, no fall in pigment production and no reaction whatever to the feeding of bile pigments.

A study of the *bile pigment output of the Eck fistula liver* furnishes some interesting facts to consider at this time. Dogs with combined

Eck and bile fistulae eliminate less bile pigment than controls—sometimes but 30 to 50 per cent of normal (60). The Eck fistula liver is functionally inefficient and there is no direct contact with the portal blood. Both these factors may well contribute to this low pigment output but the main point to emphasize is that the pigment output is influenced by *liver function* rather than by the amount of hemoglobin waste products formed in the body. We have ample evidence that various liver injuries will likewise depress bile pigment excretion—again clear evidence that the liver has a constructive function in producing bile pigments rather than a simple passive eliminative function.

Certain conditions in bile fistula dogs will cause or be associated with a maximal bile pigment elimination—for example, a combination of splenectomy and anemia (19). Such periods of maximal bile pigment formation are of very great interest and deserve careful study as such investigations may lead to a more complete understanding of the *fundamental pigment stimulus* in the body. This is of obvious interest to physiologist and clinician alike.

One last point concerning pigment formation invites speculation. We recall that vessel endothelium (including Kupffer cells) and mesothelium (lining cells of serous cavities) can rapidly transform hemoglobin into bile pigment. Further, the embryo endothelium in the chick is able to develop red blood cells containing hemoglobin (47). We recall the embryonic hematopoietic function of the liver containing hepatic epithelium and Kupffer cells. We have pointed out the intimate relationship between the two types of cell normally concerned with bile pigment production (hepatic epithelium and Kupffer cells). The degradation of hemoglobin to bile pigment concerns two important cells (liver epithelium and Kupffer cells). May these two cells have any capacity for reversing this reaction and may they not be concerned with hemoglobin construction or the production of parent substances used by the marrow cells which put the finishing touches on the hemoglobin of the erythrocytes? There are many suggestive things in animal experiments but convincing proof of this point is not yet at hand although at times it seems to be almost within one's grasp.

UROBILIN. It may be debatable as to whether urobilin is a normal constituent of bile. However, it is claimed by some investigators that it is present in normal human bile (53). These claims are based on analyses of bile obtained by the duodenal tube and obvious objections may be raised to these conclusions as the bile was in contact for

a short time at least with the intestinal mucosa and its intestinal flora. Urobilin is constantly present in fasting bile-fistula dog bile (62). Urobilin has been observed frequently in human bile under abnormal conditions (Wilbur and Addis (64) and others). It has been suggested by Brulé and Garban (4) that urobilin is formed directly from the hemoglobin by a reaction which goes on in the circulating blood and living tissues. However they give no experiments to support this very interesting suggestion as it is obviously necessary to exclude the participation of the liver. Quadri (41) submits a series of experiments to show that *in vitro* the common bacteria cannot change hemoglobin into urobilin.

We must refer to a fundamental error which is unfortunately firmly fixed in the minds of clinicians and laboratory workers—the belief that stercobilin or urobilin is absorbed from the intestine. We know of no evidence of any sort to indicate that urobilin or bile pigments are absorbed from the intestine, once we grant that urobilin may be present at times in the bile within the bile ducts. We believe that urobilin is formed in the liver, particularly in abnormal conditions of the biliary tract (cholangitis) but know of no evidence that it is absorbed from the intestine—refer to figure C.

All the evidence at hand indicates that the pigment substances in the bile (bilirubin, biliverdin and urobilin) subsequently serve no useful purpose and are true excretory substances. There is no evidence for any “circulation” of the bile pigments and when this word is used it should be limited to the bile salts which are so rapidly absorbed from the intestine.

**BILE SALTS.** In this discussion we may concern ourselves with glycocholic (including glycocholeic) and taurocholic (including taurocholeic) acids which are found in the bile as the sodium salts. If our ignorance about the complete story of the bile pigments is disturbing, then our lack of understanding as to the source and internal metabolism of the bile acids is pathetic—this in spite of much careful study and investigation. The most important fact to date is the “circulation” of the bile acids; by which we mean a rapid absorption of the bile salts from the intestine with prompt reappearance in the bile. This fact was established very early in the study of bile physiology (49) yet we understand nothing as to the control of this reaction in the body. We may be led into a ridiculous position if we choose to argue from the few facts established by experimental work—for example, a bile-fistula dog excretes considerable amounts of bile acid during fasting periods,

therefore the substance is produced constantly in the body. Again we find good evidence that bile acids by mouth in bile fistula dogs are promptly and quantitatively eliminated in the fistula-bile—for example, 1 gram bile salts by mouth will be *excreted quantitatively in the fistula bile within 6 hours*. In the normal dog whose bile escapes into the duodenum we should indeed find an amusing condition—new bile acids formed daily and all the bile acids poured into the intestine *quantitatively* absorbed—a vicious circle with rapid transformation of the dog into a pillar of bile salt! Evidently there is a mechanism in the normal animal which controls the production and destruction of bile salts within certain limits but this is a complete mystery.

One great difficulty was the lack of accurate and rapid methods for bile acid analysis and the older work required large amounts of bile, much time and expensive chemical extractives. The new method of Foster and Hooper (12) is a great improvement and permits of rapid and accurate analysis of taurocholic acid in small amounts of dogs' bile. They give a critical review of the older methods. Their method consists of hydrolysis of taurocholic acids into cholic acid and taurin. The amino nitrogen of the taurin is then determined by the gasometric method of Van Slyke. A further modification of this method by Schmidt and Dart (48) enables the investigator to determine accurately the two separate bile acids, taurocholic and glycocholic. They find complete absence of glycocholic acid in dog and sheep bile, a preponderance of glycocholic in pig's bile and approximately an equal division between the two acids in human fistula bile.

We may now ask what information has been obtained from fistula animals as to bile acid production. For a detailed discussion of these factors we wish to refer to the papers by Foster, Hooper and Whipple (13). Bile fistula dogs are not wholly satisfactory but are the best experimental animal at present and most of the accurate information is obtained from observations on these dogs.

Under normal conditions of health there is great variation day by day in the output of bile salts. A part of this variation is due to diet factors, and in general the bile salt excretion is much greater on a meat diet than on a carbohydrate diet. The actual curve of bile acid secretion as related to food intake is not easy to determine because of factors other than food which influence bile acid production. For example, it has been noted repeatedly that after a fasting period of 2 to 4 days or longer the prompt rise in bile acid production following a meat feeding may not appear until after the second or third feeding. This

suggests a fasting depletion of a reserve material which is made up before the customary rise in bile acids appears after meat feeding.

During fasting periods there is a uniform excretion of taurocholic acid in bile fistula dogs. This level of excretion will be lowered somewhat by sugar feeding and we note an interesting parallel between the bile acid excretion and the urinary nitrogen excretion. Of course this suggests an important relationship between body protein metabolism and bile acid metabolism. Evidently there is an important *endogenous factor in bile acid metabolism*.

Within limits, an increase in food protein will reflect an increase in bile acid excretion. The level of bile acid excretion can be raised to a maximum by meat feeding (exclusive of bile salt feeding). This fact suggests that there are important *exogenous factors in bile acid metabolism*.

The Eck fistula liver furnishes us with very important data: This type of liver is known to be functionally subnormal and it produces about one-half the normal amount of bile acid on a standard diet. This is important evidence that the production of bile acids depends at least in part upon the functional capacity of the hepatic epithelium. It is generally accepted that bile acids are formed only by hepatic epithelium but the evidence for this is fragmentary and, when critically reviewed, none too convincing. The Eck fistula liver furnishes evidence to support the accepted belief and other experiments will be published soon to give further confirmation. These experiments (Smyth and Whipple) will show a remarkable depression of bile salt excretion due to *small doses* of poisons known to act specifically upon hepatic epithelium.

Bile acids can be readily separated into their constituent parts: taurochloric acid = taurin + cholic acid and glycocholic acid = glycocoll + cholic acid. Both these amino acids are present in the body and glycocoll can be formed within the body. Taurin is derived probably in large part from the cystein of the food or body protein. Under usual diet conditions there is probably an excess of glycocoll and taurin available in the body to satisfy any demands made in the bile acid synthesis.

*Cholic acid* is the determining factor in this reaction and we may as well begin with the statement that almost nothing is known about the origin of cholic acid in the body and the disposal of any excess of this compound other than in the bile. Cholic acid has a structural formula which is rather complex but contains no nitrogen. Pregl (40) states

that cholic acid is a hexahydroxybenzene and structurally related to turpentine and camphor. A considerable literature on this subject has been recently reviewed by Foster, Hooper and Whipple (14). In that paper sufficient evidence is submitted to prove that no physiological relationship exists between camphor and turpentine and cholic acid.

The same holds for cholesterol which has often been suspected as the precursor of cholic acid (2), (65), but careful experiments negative this interesting suggestion. In the same series of experiments (14) it was shown that red cells fed by mouth or hemolyzed and introduced intravenously had no influence on the bile acid excretion. A recent paper by Beth (2) reviews the suggestion that bile acids are derived by the disintegration of cholesterol. He claims that this interesting reaction takes place within the Kupffer cells but we were unable to locate the data to establish this claim. Further objection to this work may be made on the grounds that the material is obtained by the duodenal tube. One must be guarded in the study of this material as analyses can be at best qualitative, as we are dealing with unknown dilutions and confusing additions of gastric and duodenal secretions. We may conclude that *cholic acid is the limiting factor which determines the level of bile acid excretion in fistula bile but the origin and fate of cholic acid are obscure.*

In this connection we may note that taurin intravenously has no effect on the excretion of bile acids(14). Taurin plus cholic acid by mouth causes a notable cholagogue action and increase in bile acid output just as taurocholic acid by mouth. Evidently these substances have a strong physiological attraction within the body for we note the same effect if the cholic acid is given by mouth and the taurin intravenously. Cholic acid fed during long fasting periods gives a minimal output of bile acids but fed during full diet periods gives a maximal output of bile acids with the usual cholagogue action. We believe this reaction is to be explained by the available supply of taurin within the body which is at a low level during fasting but not during full diet periods.

The bile acids are generally believed to be formed only by the activity of the hepatic epithelium, as stated above. These substances are looked upon as true secretory factors as they serve a useful purpose in the digestive process within the intestine. We recognize a significant influence exerted by the bile acids upon fat digestion and intestinal putrefaction. It would be of great interest to learn whether the bile acids are con-

cerned with any of the endogenous fat metabolism reactions which take place within the liver. There are scores of interesting possibilities connected with bile acid metabolism but space does not permit such discussion.

**DISSOCIATION OF BILE CONSTITUENTS.** Stadelmann was the first to call attention to this interesting fact. He emphasized the versatility of the hepatic epithelial cell. It is very easy to give examples in which one or the other of the bile constituents may be increased without equivalent increase in other constituents. Hemoglobin given intravenously will cause a rise in bile pigments but no significant change in bile salts. Taurocholic acid by mouth will cause a great increase in total bile flow and bile acid output but the total bile pigment may be decreased. Taurocholic acid plus sugar by mouth may show no increase in volume but great increase in bile acids. This experiment probably shows the maximum power of the liver to concentrate bile acids in bile and the concentration may rise to 7 or 9 per cent by weight. The water elimination in the bile is obviously influenced profoundly by sugar or carbohydrate feeding. Meat feeding will be associated with a high bile acid output and a lowered bile pigment excretion. Instances need not be further multiplied. Much less is known about the fluctuations of the other bile constituents and such data are very much to be desired.

**CHOLAGOGUES.** The chief if not the only real cholagogue is *whole bile* or its active principle—that is, the *bile acid* fraction. The mere fact that drugs are claimed to be cholagogues by some and denied by others will indicate to the student of bile physiology that the cholagogue action is at least insignificant if not absent. We may note the few points about which all workers are in accord. Bile salts by mouth or intravenously will cause a distinct rise in the volume of bile secreted by a temporary or permanent fistula. Likewise a meat diet favors an abundant flow of bile in a bile fistula dog but a diet rich in carbohydrates, especially sugar, will decrease the bile flow under similar conditions. That *sugar has a distinct inhibiting influence* on the bile flow can be shown by combining bile salts and sugar by mouth. This procedure will give a maximal concentration of bile acids in the bile but little or no increase in the volume of bile. This shows the importance of diet as influencing the bile flow.

The duration of the cholagogue effect of bile salts depends upon the dosage. With a dose of 1 to 2 grams taurocholic acid the cholagogue action will be over 4 within 4 or 8 hours and the ingested acid can be

recovered quantitatively in the fistula bile. Larger doses of taurocholic acid (8 to 12 gms.) by mouth may prolong the cholagogue effect for 24 to 48 hours or even longer. It is generally admitted that glycocholic acid has a less powerful cholagogue effect than has taurocholic acid.

What shall we say of the various drugs advocated as cholagogues by certain investigators? We believe the attitude of Stadelmann is correct—that one must be critical of claims not supported by uniform and distinct increases in bile flow such as may be produced by bile feeding. None of the so-called cholagogues stand this test except, as stated above, the bile salts (especially taurocholic acid). The salicylates have many advocates as cholagogues (25), (37) and as many report negative results. The writer has never observed any constant reaction to salicylates in bile fistula dogs. Neubauer (36) and others have studied the effect of pilocarpin, adrenalin and atropin. We find no uniformity of reaction or opinion. The changes noted are not striking and may or may not be due to the action of the drug on the liver epithelium. Large doses of adrenalin are reported (36) to cause fall in volume excretion. The same indefinite reactions are reported by Weinberg (55), Okada (37) and others following administration of dilute acids, soaps, salts, glycerine and albumoses. Downs and Eddy (7), (8) report minor fluctuations in bile flow due to a host of organ extracts, adrenalin, secretin, etc. Eiger (10) claims to demonstrate secretory fibers in the vagus which influence bile flow from the liver. The changes are minimal and the writer is not convinced that the changes can not all be explained on the basis of simple vasomotor reactions.

We have considerable evidence that certain drugs can relax the bile papilla sphincter and perhaps also cause certain constriction of the bile ducts and gall bladder. For example, Doyon (9) showed that duodenal irritation might cause relaxation of bile papilla and constriction of gall bladder and ducts. Meltzer and Auer (32) emphasized this reaction as caused by magnesium sulfate and recently this has been applied to the clinical study of disease (30), (50). Much information of value is to be expected from a careful study of normal and diseased humans by this method. There is need, however, for critical analysis of these data to guard against too free interpretation of results.

All physiologists who have worked with bile fistula animals under anesthesia or shortly after an operative procedure are all too familiar

with periods of temporary inhibition of bile flow. Such periods may last for hours and seriously interfere with the experimental procedure. The cause for this is not understood although it is believed by many that operative manipulation of the gastro-hepatic omentum and nearby structures will predispose to such a condition. Whether there is such a thing as spasm of the smaller bile ducts or actual reflex inhibition of the hepatic epithelial secretion must for the present be left an open question.

In conclusion we may repeat that the only active cholagogues are bile or bile salts. A number of drugs are proposed as cholagogues by a host of investigators but when we examine the experimental data we find minimal fluctuations caused by a variety of drugs—salicylates, chloral hydrate, soaps, acids, albumoses, etc., etc. If cholagogue action is present it is slight, or delayed or transient and is usually less than can be demonstrated as due to food factors (meat, for example). Such cholagogue reactions fade by comparison with the reaction caused promptly by bile salts. This reaction is constant and lasting and of striking volume. When claims are made that a substance is a cholagogue it should always be measured by the standard of the only known active cholagogue (bile salt) and to date all such substances fail when so tested.

**CHOLESTEROL, PHOSPHATIDS, FATS, SOAPS:** *Cholesterol* appears to be the most important member of this group and this is to be explained in part by the fact that cholesterol is such an important constituent of gall stones. The formation of biliary calculi is of much concern to physiologist, pathologist and surgeon. Experimental work with cholesterol will be greatly furthered by an accurate, rapid and simple analytical method but as yet this method is not available. The bile acids, bile pigments and lecithin are sources of trouble in adapting methods to cholesterol analysis in bile.

Suggestions as to the source of cholesterol may not as yet have exhausted all the possibilities but their number is legion and the writer will mention but a few. Stadelmann (51) suggests its origin from the bile duct epithelium and degenerating liver cells. He could find no evidence for increase in the bile following cholesterol feeding. On the contrary, Fasiani (11) reports an increase in bile cholesterol in bile fistula dogs after intravenous injection of large amounts of cholesterol. It is fair to say that the rise is only from an average of 5 mgm. per day to about 13 mgm. per day after a dose of 1.7 grams intravenously. It is obvious that only a very small part of the cholesterol so given is

excreted promptly in the bile. Fasiani claims an important relationship between the blood and bile cholesterol—any excess in the blood being excreted in the bile. We cannot see the evidence to establish this point.

Feeding experiments by D'Amato (6) give similar results but he uses ox brain and eggs in the food to give the excess of cholesterol—about 5 to 6 grams cholesterol per day. This great excess in the food increases the bile cholesterol from 6 mgm. to 8 mgm. per day and he states that bile is evidently not the chief avenue of elimination for cholesterol in the food. Stepp (52) placed rats and dogs on a lipid-free diet for a number of weeks and records a fall in bile cholesterol to 10 or 15 per cent of normal found at autopsy. It may be objected that these very abnormal diets caused systemic abnormalities which were responsible for this low cholesterol level rather than the immediate lack of cholesterol in the food. Some very suggestive experiments are recorded by Gardner and Fox (15). They report in man on standard diets an *excess of excretion over cholesterol intake*. Cholesterol is obviously synthesized in the body if these observations are correct and this work should stimulate further investigation in this field. It would be very desirable to know whether this excess excretion is present both on diets poor in cholesterol as well as on diets rich in cholesterol.

Among others Chauffard, Laroche and Grigaut (5) have suggested control of cholesterol metabolism by the adrenals. They believe that the adrenals are the sources of cholesterol and control the body supply. It would be somewhat difficult to disprove this possibility but the writer can find no proof furnished by the authors that the adrenals control cholesterol metabolism and the proof should come from the proponents of this hypothesis. Endocrine control of every body function may be a fact but much of the present enthusiasm for such explanation unfortunately has little or no ballast of solid fact. This type of ballast is sadly needed when one embarks on the uncharted sea of endocrinology.

We find repeatedly the statement that cholesterol is the precursor of the bile salts, as noted above. Lifschutz (28) goes so far as to claim that bile acids are derived quantitatively from cholesterol. Such claims are usually made by chemists who are impressed by the close chemical relationship which is known to exist between cholesterol and the bile acids. Windaus (65) for example shows that an identical split product can be produced by hydrolysis of the two substances. Physiological experiments do not support this claim and in fact exclude

the possibility that bile salts in body metabolism are derived from cholesterol. The experiments of D'Amato (6) and Foster, Hooper and Whipple (14) are convincing.

The cholesterol content of human bile is given by Nathan (35) and Rosenbloom (44). There are very wide variations both in health and disease. Human fistula bile contains 0.07 to 0.10 per cent cholesterol. Human bile at autopsy is very different in content and may vary all the way from 0.06 to 1.06 per cent. The variations are as great in cases with normal liver findings as in cases with liver and biliary tract disease. In the interpretation of analyses of gall bladder bile one must not forget the remarkable concentrating power of the gall bladder, therefore casual analyses of such bile can have relatively little significance as to quantitative values. Cholesterol excretion has been studied by means of the duodenal tube but there are serious limitations to this method when applied to quantitative analysis.

To sum up, we may give the few facts which relate to cholesterol metabolism. Lipoid-free diets may be associated with low bile cholesterol values. Excess feeding of cholesterol gives but trivial increases in bile cholesterol. The elimination of cholesterol in man may exceed the cholesterol intake, indicating a production of this substance in the body. The cholesterol content of human bile varies within wide limits and one can determine no uniform relationship to disease. It is possible that diabetes and pregnancy are exceptions to this statement. The evidence is very strong that there is no physiological relationship between cholesterol and bile acids. It is suggested by various writers that cholesterol results from secretion of the biliary tract epithelium, from liver parenchyma degeneration, from red cell disintegration, from general tissue wear and tear, from food cholesterol, from the adrenals and other glands of internal secretion, etc. Nobody has as yet suggested that cholesterol is derived from the wear and tear of the cerebral cortex under the stress of environmental conditions!

*Lecithin* has been found in specimens of bile analyzed by chemists. This applies to human and animal bile. Rosenbloom (44) gives figures for many such analyses. Lecithin is reported usually as less in percentage content of the bile than given for cholesterol but Rosenbloom finds more lecithin than cholesterol. The values given for human bile vary from 0.05 to 6.4 grams per 1000 cc. bile. The bile of herbivora appears to contain less lecithin than does human bile (43). Extraction and analysis of lecithin in bile is very difficult. Long and Gephart (29) have emphasized the tenacious union which exists between bile

acids and lecithin and it is very difficult to separate these two substances by extraction methods.

Jecorin has been found in bile but recent work of Levene indicates that this substance is a mixture of two phosphatides, lecithin and cephalin. Levene (27) reviews evidence that lecithin from the liver is different structurally from egg lecithin but this liver lecithin may or may not be identical with the bile lecithin. The writer perhaps has made it clear that there is plenty of work yet to be done before an understanding of the origin and significance of lecithin in the bile may be approximated.

*Soaps and fats* are always reported as present in chemical analyses of bile. In many analyses we find the ether extractions lumped together. We know of no experimental observations to give information as to normal or abnormal fluctuations in the fat or soap content of the bile. Few if any suggestions as to the source of such substances have been made by the workers in this field. We may conclude that our ignorance on this subject is approximately 100 per cent.

**MINERAL CONSTITUENTS.** So far as is known, the salts are mainly chlorides of sodium and potassium, and phosphates of calcium, magnesium and iron. There are traces of copper and traces or no sulphates. Inorganic substances in human bile are given as 0.6 to 1.1 per cent, but concentrated bladder bile may be much higher in salt content.

*Iron* elimination in the bile has been much studied and it is fair to say that there are wide differences of opinion. Some workers deny the presence of iron in bile but they are in the minority. Some workers claim that iron elimination is much influenced by food iron intake. Leone (26) claims that in bile fistula dogs the normal level of iron secretion (4.2 to 8.2 mgm. per 100 cc. bile) can be raised on a diet rich in iron in protein combination to a level of 22.8 mgm. per 100 cc. bile. He finds less increase on subcutaneous administration and a negative reaction on giving inorganic iron by mouth. The body metabolism of iron is certainly not completely understood and information is very much to be desired. A study of the causes of fluctuation of iron excretion in the bile will surely give information of much value.

Excretion of the *chlorides* and *phosphates* in the bile has been very little studied but there is evidence that these salts in the bile have some significance. We may give an illustration. Bile fistula dogs under observation for many months very often develop *bony abnormalities*. The essential feature of this condition appears to be a loss

of inorganic salts from the bones with a great thinning of all bones, and spontaneous fractures of ribs and long bones. This indicates a great loss of salts from the body presumably in part through the bile and a lack of power of assimilation. We hope to report the complete mineral metabolism of these dogs in the near future. Another of the many curious factors concerned in this condition is the observation that the addition of cooked liver to the usual diet will prevent the development of this condition. The main point we wish to make is that the bile excretion in bile fistula dogs may be concerned with the mineral metabolism of the body and bony framework.

**MISCELLANEOUS.** *Urea* is always present in bile and usually in the same concentration as found in the blood and tissues. It is probable that its uniform distribution in all the body protoplasm and fluids is sufficient explanation for its presence in the bile.

*Enzymes* of various sorts are reported as present in the bile and these may or may not be associated with the activity of the specific hepatic or biliary epithelium.

*Ethereal sulphates* and *glucuronates* are found in bile but the reason for this is not understood. It is probably related to the recognized conjugating power of the liver epithelium (38).

*Mucin* is present in varying amounts in bile and is thought to be a secretion of the gall bladder epithelium. This substance is a nucleoprotein. The significance of this substance in the bile is not understood.

**GALL BLADDER AND BILE DUCTS.** The interrelation between the gall bladder, the bile ducts and the flow of bile has been the subject for many dissertations. It was recognized that bladder bile was much more concentrated than duct bile but it was debated whether this was due to absorption of water in the gall bladder or the addition of solids by the bladder epithelium. Rous and McMaster (45) have answered some of these questions by means of carefully controlled experiments. They show a remarkable power of bile concentration inherent in the gall bladder which in the dog can concentrate whole bile to  $\frac{1}{5}$  or  $\frac{1}{10}$  the original volume during a 24-hour period. Simple passage through the gall bladder may concentrate whole bile to  $\frac{1}{2}$  or  $\frac{1}{4}$  its original volume. Rous and McMaster (46) further studied pure secretion of hepatic duct epithelium and found it to be water clear, of low specific gravity, containing traces of cholesterol.

Periodic discharge of bile from the gall bladder has been assumed but little studied. Recently experiments have been performed to throw light on this question by the use of dyes introduced into the gall bladder

(1). In dogs it was found that irritation of duodenal mucosa called out a flow of duct bile but *not gall bladder bile*, as has been assumed by many investigators using the duodenal tube and bile analysis. Stimulus of various nerves and even the wall of the gall bladder called forth no response and outpouring of bladder bile. Dyes introduced into the gall bladder were found after 1 to 3 days but not after 7 days. All this indicates a slow and very irregular filling and emptying of the gall bladder and our knowledge as to the controlling factors is obviously very fragmentary.

**BILE SECRETION.** The flow of bile in most animals with or without gall bladders is fairly continuous. There are periodic fluctuations in bile flow and bile production which may depend upon sphincter control of the bile papilla, stimulation of the duodenal epithelium, food factors, nerve stimuli, vascular changes, etc. When an investigator attempts to determine which single factor is concerned in any given bile flow reaction there is indeed need for caution and guarded conclusions.

The amount of bile produced varies with many factors—known and unknown. Much recorded data from human bile fistula cases is available (Stadelmann (51), Pfaff and Balch (39) and others). The volume in such cases may vary from 500 to 1000 cc. per 24 hours. A great amount of data on bile fistula dogs is available and some of these facts have been reviewed recently by Wisner and Whipple (66). Collections of bile for 24 hours in 6-hour samples show a number of interesting details. One is surprised to note little if any decrease in bile flow during night periods. These bile fistula dogs show no constant variation in bile pigment excretion during the different 6-hour periods. There are considerable variations in pigment output but the reasons for such variations are not clear. Bile acid production is much higher on a meat diet than on carbohydrate feeding, as noted above. On a meat diet one notes a slight falling off in bile acid secretion during some of the night periods but this is not so evident in the carbohydrate feeding experiments. For these dogs the volume output per 24 hours averages between 10 and 20 cc. per kilo body weight.

The *secretion pressure* of the bile is low in all animals. Values of 210 to 375 mm. bile are recorded for dogs and cats by Herring and Simpson (16) in acute bile duct obstruction. Similar values are reported by Mitchell and Stifel (34) in cats and dogs after chronic obstructions of 2 to 6 days. Similar observations in human patients have been recorded by Robitsek and Turolt (42). The pressure

in such cases was 210 to 270 mm. bile. Various drugs like atropin or pilocarpin have little or no effect upon this level of secretion pressure. Evidently there is a pressure level (commonly 210 to 300 mm. of bile) at which bile secretion and bile absorption are in equilibrium.

This obstruction pressure does not in any sense inhibit the production of bile and it is common knowledge that the bile constituents promptly appear in the blood and urine. This results in a clinical condition of icterus which does not concern us at this time. We may inquire as to the escape of the bile constituents from the bile passages in biliary obstruction. Mendel and Underhill (33) gave experimental evidence that dyes and chemicals injected into the bile passages escape by way of the blood stream and only incidentally by way of the lymphatics. Whipple and King (63) gave proof that the bile pigments in biliary obstruction escape promptly by means of the blood stream and appear in the urine. This reaction was not modified by a thoracic duct fistula and the bile pigments appeared more promptly in the urine than in the thoracic duct lymph after a biliary obstruction in the dog. We may conclude that during biliary obstruction the absorption of the bile constituents is effected mainly by the liver blood capillaries and only to a slight extent by the lymphatics.

**BILE FLOW NECESSARY FOR LIFE?** The question as to the necessity of bile to normal life and function may be answered in the affirmative. There may be doubts in the minds of physiologists and clinicians as to whether obstruction of bile or deviation of bile from the intestine to the exterior (fistula) will invariably give fatal results. We believe the evidence is becoming more convincing that bile is a necessary life factor. To be sure we observe human beings with long-standing obstruction who live months and "die of cancer." But it is not easy to state the cause of death in such cases nor simple to predict what would have been the story in the absence of the tumor complication.

Bile fistula animals appear to tolerate the exclusion of bile over considerable periods of time without serious impairment of health. Diet is an important factor in this equation. For example, a fistula dog will usually die within 2 months with acute intestinal disturbance if kept on an ordinary diet of kitchen scraps. A diet of milk, cooked potatoes, rice and bread will prolong life considerably and such dogs may live in good condition for 4 to 10 months and die with advanced bony abnormalities, as noted above. Abnormal pigment disturbances and true purpura with fatal hemorrhage may develop in such dogs. Addition of cooked liver to the above diet will usually improve the

condition and prolong the period of health in these fistula dogs but they are apt to suffer at times from some of the conditions noted above.

Bile fistula dogs with tiny fistulous tracts connecting with the duodenum may live for long periods in perfect health and function. The amount of bile gaining entrance to the duodenum is very small and can scarcely exceed 10 or 20 per cent of the total flow which escapes through the external fistula. This shows how little bile may serve to change the entire clinical picture from abnormal to normal. The same amount of bile by mouth will not have any similar effect.

We feel safe in concluding, with certain minor reservations, that secretion of bile into the intestine is necessary for normal health and even for actual continuation of life beyond a few months' period.

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## NON-PROTEIN NITROGEN OF BLOOD IN HEALTH AND DISEASE

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It has been known for nearly a hundred years that patients suffering from Bright's disease must be poisoned by their own waste products. These patients often passed little or no urine, and from them emanated the characteristic odor of decomposed urine, so that it did not require any unusual degree of scientific imagination to recognize the fact that the blood and tissues of these subjects must be loaded with the materials which normally find their way into the urine. For many decades the subject, nevertheless, did not progress very far beyond the concept conveyed in the term *uremia*. All attempts to show that the uremic coma is due to any specific urinary ingredient, such as urea, ammonia, potassium or the "extractives," were failures; nor did the investigations purporting to elucidate such specific causal connections particularly advance the available knowledge concerning the chemical composition of blood in Bright's disease, in other diseases, or in health. The early chemical proofs that normal blood contains urea were necessarily crude and would probably never have been accepted as proofs at all, if it had not been for the fact that the presence of urea was on *a priori* grounds so probable that no one seriously questioned it. Such tests as the identification of urea nitrate crystals (Schmidt, 1846), or the change in form of sodium chloride crystals produced by the presence of urea (1840-1855) were, of course, not capable of advancing the subject.

It is scarcely worth while to try to sift the intricate mixture of illusion, correct observations, and correct guesses which is represented in the early literature alike of the clinical investigators and the metabolism workers. Their reasoning power was necessarily much superior to their analytical skill or their meager laboratory facilities, and their analytical data could have been little more than mere ornaments attached to good, logical conclusions. The figures for some constituents might be fairly accurate; reasonably plausible figures for the urea content of different kinds of blood were published as early as 1850 to 1860.

The correctness of the concept that waste products must accumulate in the blood when the kidneys fail to perform their function in the formation of urine was established as early as 1821, when Prevost and Dumas showed that extirpation of the kidneys was followed by a gradual increase of the urea content of the blood—a finding repeatedly verified by other well-known investigators during several following decades.

Many recognized, of course, that little progress could be made on the basis of qualitative tests for urea or other urinary constituents in the blood, but it was one thing to see the need of suitable methods and quite another to find them. The earliest quantitative methods, borrowed from the field of urine analysis, such as weighing the isolated urea nitrate, or Liebig's mercuric oxide titration, or the laborious CO<sub>2</sub> method of Bunsen, were elusive tools in the hands of early nineteenth century clinicians. Kjeldahl's method for the determination of nitrogen did not come out until 1883, and before the discovery of that method there can scarcely be said to have existed the possibility of acquiring any comprehensive information concerning retention or the normal levels of the non-protein nitrogenous products in blood. The older Knop-Hüfner hypobromite method, in the form of innumerable modifications, was assiduously applied for many years after it had been abandoned by the chemists. This method is in fact still used in some places, notably in France. And, inaccurate as it is, it doubtless did good service, at least to the extent of keeping up interest in the problem of urea retention in nephritis. Widal's work is still largely based on urea determinations obtained by means of the hypobromite process.

While Kjeldahl's ingenious method supplied a sound basis for reliable determinations of the nitrogen, not only in blood, but in all kinds of biological material, its application to the study of the non-protein nitrogen in blood was neither particularly extensive nor particularly fruitful until Strauss (1) in 1902 made his well-known attempt to classify the various forms of nephritis partly on the basis of the amounts of non-protein nitrogen found in a large number of such patients. Strauss worked with blood serum. This comparatively early contribution of Strauss is now perhaps only of historical importance, as it has been replaced by more detailed and accurate classifications, such as those of Volhard (2), in which due attention is given to the rest-N of the blood. A detailed account of these important studies of various forms of nephritis does not come within the scope of this article because they are predominantly clinical.

The expression "non-protein nitrogen" has of late replaced the earlier term "uncoagulable nitrogen," while some writers have used the non-descript expression "rest-nitrogen." The terminology used is as yet of minor importance because it is not at present possible to describe all the nitrogenous products present in the filtrates used for analysis; and those products, moreover, differ to an appreciable extent according to the methods used for removing the colloidal protein materials. These differences in the total non-protein nitrogen of filtrates obtained by different methods of removing the albuminous materials are interesting, for all the methods in common use do remove the coagulable proteins and do leave in the filtrate the common waste products (or at least the urea), as well as the amino acids. The different values given by different processes for obtaining the filtrates indicate, therefore, that there are present in blood some products which are partly thrown down together with coagulable proteins and partly escape precipitation, and that the extent to which this is the case depends on the character of the method used for precipitating the proteins.

Abel (3) has obtained results indicating that these unknown products give certain protein reactions, and he suggests that they are peptones; while Folin and Berglund (4) have pointed out that since the products are more abundant in corpuscles than in plasma the products may belong to the histones. Neither of these interpretations covers the important fact that the unknown nitrogenous products in blood filtrates are greatly increased in bloods in which there is excessive retention of nitrogen, for in such bloods the undetermined nitrogen is even more abundant in the plasma than in the corpuscles. These facts are referred to here only to emphasize the point that it is not possible at present accurately to define the nitrogenous materials contained in the blood filtrates by means of which we study the "uncoagulable nitrogen" the "non-protein nitrogen" or the "rest nitrogen" of blood. The products contained in these filtrates can be classified in three groups:

A. The nitrogenous waste products.

B. Absorbed nitrogenous food materials.

C. Undetermined materials, including some undetermined waste products, some undetermined absorbed food products, and in addition some products of unknown origin.

It may be pointed out that the modern interest in the non-protein nitrogen of blood as an essential factor in the interpretation of normal protein metabolism was revived about the same time as the clinical importance of nitrogen retention began to take on added interest through

TABLE 1\*

*Sample analyses of protein-free blood filtrates obtained by means of tungstic acid*

NUMBER	MGM. PER 100 CC. BLOOD					
	Total N	Urea N	Uric acid	Preformed creatinine	Total creatinine	Sugar
1	26	10	1.3	1.5	6.0	89
2	26	13	1.0	1.4	5.3	100
3	28	12	1.1	1.2	6.7	98
4	28	12	2.2	2.0	5.7	83
5	29	13	3.3	1.5	6.0	86
6	29	11	2.6	1.4	5.2	95
7	29	13	1.6	1.4	6.0	85
8	30	13	2.4	1.6	5.5	82
9	30	14	4.1	1.7	5.3	82
10	32	15	2.8	1.6	5.4	91
11	32	15	3.4	1.4	5.3	97
12	32	13	2.4	1.7	6.0	104
13	33	17	2.0	1.3	4.8	83
14	33	16	2.5	1.6	5.7	105
15	33	15	1.1	1.6	5.5	95
16	34	16	0.8	1.3	6.1	119
17	34	16	2.6	1.5	5.9	106
18	35	17	2.1	1.6	6.0	89
19	35	17	2.0	1.4	5.5	77
20	35	18	2.0	1.7	5.7	86
21	35	18	2.9	1.6	5.8	95
22	35	17	3.2	1.4	5.5	94
23	35	18	2.5	1.5	6.0	89
24	35	19	2.2	1.5	5.3	91
25	35	22	3.5	1.4	5.7	87
26	35	17	2.3	6.7	6.7	83
27	35	18	1.6	1.3	6.5	104
28	36	17	2.8	1.5	5.2	100
29	37	18	2.1	1.5	5.5	94
30	38	18	2.2	1.7	5.4	95
31	39	18	2.6	1.8	6.7	103
32	39	18	2.9	1.5	6.0	87
33	40	18	2.0	1.6	6.0	98
34	40	20	2.6	1.7	5.6	95
35	41	19	4.8	1.5	5.9	93
36	41	19	4.2	2.5	6.6	109
37	43	19	2.2	1.7	6.3	78
38	139	106	5.4	12.5	19.4	99
39	147	115	8.9	11.0	20.5	170
40	275	237	14.3	13.6	27.2	157

\* Folin and Wu: Journ. Biol. Chem., 1919, xxxviii, 109.

TABLE 2\*

*Normal minimum, maximum and average blood content of non-protein nitrogenous products (from 12 young men)*

	WHOLE BLOOD PER 100 CC.				PLASMA PER 100 CC.				CORPUSCLES PER 100 CC.			
	Amino-acid N	Urea N	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Undetermined rest N	Total non-protein N
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
After a night's fast												
Minimum.....	5.7	8.9	10.1	27.8	4.3	9.6	1.8	18.0	6.7	7.7	18.3	37.7
Maximum.....	7.8	15.2	17.5	39.4	6.2	17.3	11.5	30.0	10.7	13.2	33.8	55.0
Average.....	<b>6.4</b>	<b>11.5</b>	<b>13.7</b>	<b>32.1</b>	<b>5.3</b>	<b>12.4</b>	<b>6.7</b>	<b>24.7</b>	<b>8.2</b>	<b>10.3</b>	<b>24.7</b>	<b>43.6</b>
After carbohydrate intake												
Minimum.....	4.9	8.0	6.4	21.0	3.5	9.2	1.8	17.0	5.9	5.2	9.0	24.8

\* Folin and Berglund: Journ. Biol. Chem., 1922, li, 415.

TABLE 3

*Illustrating substantial uniformity in the retention of the several nitrogenous waste products (Not previously published)*

case	WHOLE BLOOD PER 100 CC.						PLASMA PER 100 CC.						CORPUSCLES PER 100 CC.						vol. per cent
	Amino-acid N	Urea N	Creatinine	Uric acid	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Creatinine	Uric acid	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Creatinine	Uric acid	Undetermined rest N	Total non-protein N	
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	
1	5.0	13	1.8	3.7	19	39	4.3	13	1.8	<b>3.7</b>	20	<b>39</b>	5.9	13	1.8	3.7	18	39	45
2	6.4	49	2.0	4.5	7.4	65	5.8	47	2.3	<b>6.4</b>	18	<b>74</b>	7.2	52	1.5	1.6		51	40
3	4.5	49	3.0	4.7	22	78	3.5	52	3.0	<b>6.1</b>	18	<b>77</b>	7.4	41	3.0	0.9	31	81	27
4	5.8	60	7.7	4.8	37	107	5.4	71	8.1	<b>6.8</b>	21	<b>103</b>	7.2	26	6.5	0	86	122	23
5	7.6	91	7.2	6.4	41	144	6.2	109	8.1	<b>9.3</b>	23	<b>144</b>	11.8	38	4.4	0	93	144	25
6	8.4	174	12.9	13.6	75	267	7.5	214	14.5	<b>18.4</b>	52	<b>285</b>	9.7	113	10.5	6.1	109	238	39
7	7.3	193	16.0	12.0	48	258	7.3	234	10.2	<b>21.0</b>	51	<b>306</b>	7.3	138	11.7	0	43	193	42

the publications of Strauss. In 1902 Kutscher (5) had every reason to believe that protein is absorbed from the digestive tract in the form of amino acids, because he had previously proved that the tryptic digestion stops only with the complete transformation of protein into its component amino acids. Experimentally Kutscher nevertheless failed to find any evidence for an increase in the amino acid content of blood during active digestion, and he explained his negative results by accepting the prevalent doctrine of protein regeneration in the walls of the intestine. Cohnheim (6) found another strong reason for believing that the protein must reach the blood as amino acids when he discovered erepsin, but experimentally he also was unable to trace the absorbed nitrogenous materials into the blood. Abderhalden (7) likewise failed and explained his negative results as Kutscher had explained his. For a decade following the work of Kutscher and of Strauss more or less work was done; but very little was added to the findings reported by Strauss, and nothing to those of Kutscher. The underlying ideas survived, but experimentally the field remained unproductive.

In 1912 Folin and Denis (8) described colorimetric methods for the determination of the total non-protein nitrogen and for the urea and ammonia in blood, and a few months later Van Slyke (9) published a method for the determination of the amino acid nitrogen. In 1913 appeared the first colorimetric method for the determination of uric acid in blood; and the following year analogous methods were described for the determination of creatinine and creatine. Imperfect as the earlier forms of these micro methods of blood analyses were, their application revealed at once fundamentally important facts, which earlier investigators using improvised macro methods had sought but could not find.

In a series of papers entitled "Protein Metabolism from the Standpoint of Blood and Tissue Analysis" (1912-1914) Folin and Denis (10) traced the nitrogen of simple products, urea, amino acids, creatine and creatinine, through the walls of the intestine into the mesenteric and the portal blood, through the liver, into the general circulation, and into the muscular tissues. They also showed that the relatively high ammonia of the portal blood represented practically little else than the absorption of ammonia formed by putrefaction in the large intestine. The clearing up of this point was important in that it removed the chief experimental support in favor of the now discarded theory that the deamination process, that is to say, the hydrolytic removal of the amino groups from the amino acids, was localized in the intestinal mu-

cosa. The experimental findings of Folin and Denis have on the whole been accepted as established facts, but some of their interpretations have not fared so well. In connection with similar experiments made by Van Slyke (11) attention was drawn to the fact that while the determinations of Folin and Denis disproved the current theory of localized deamination, they did not entirely disprove the older rival hypothesis of localized protein regeneration.

By means of his now so well-known method for the amino nitrogen determination, Van Slyke abundantly covered all that was missing in the evidence of Folin and Denis. Incidentally, Van Slyke showed that the blood always contains amino acids, and furnished the first dependable figures representing this fraction of the non-protein nitrogen. In one respect Van Slyke's findings, or rather interpretations, differed from those of Folin and Denis. The localized deamination process and urea formation which had figured so extensively in the literature, particularly American literature, during the preceding decade was again revived, but was transferred from the walls of the intestine to the liver, an organ which time after time has been represented as the chief seat of the urea formation. Van Slyke's interpretation is largely based on the interesting fact that the liver takes up an excessively large fraction of ingested or injected amino acids and then loses this charge in a relatively short time (3 to 4 hours). Van Slyke's interpretation of a localized deamination and urea formation remained without serious contradiction for a number of years.

Very recently, however, Folin and Berglund (12) have advanced another explanation of the temporary storage of amino acids in the liver, observed by Van Slyke, and have obtained a fresh series of analytical data on the amino acid absorption and the urea accumulation, which, in their opinion, indicates that the urea formation is a function of all mammalian tissues, and is not predominantly localized in the liver. Their evidence is based chiefly on data showing that the accumulation of urea in the general venous blood never precedes the accumulation of amino acids.

The question of the liver versus all the tissues in the body as the seat of the urea formation is of some importance clinically, quite apart from its place in the science of metabolism. "Liver function" tests in cirrhosis of the liver based on diminished urea formation or excessive amino acid excretion will stand in need of careful scrutiny and revised interpretation, if the liver is not normally the chief seat of the urea formation.

*Ammonia.* In its major aspects the character of the normal amino acid metabolism and its relation to the phenomenon of nitrogen equilibrium have been seemingly definitely settled by means of the modern methods for detailed analysis of the non-protein nitrogen. But new problems are constantly coming in to take the places of the old. According to the deamination process there should be no difficulty in getting a definite and true picture of the ammonia formation and its function to neutralize such acids as are formed and need to be neutralized in the tissues. If deamination and urea formation is not a localized process, there should be no occasion for any localization for the neutralization of acids by ammonia.

The results which have been obtained in determining the ammonia nitrogen fraction of the non-protein nitrogen have not been in good agreement with what one might expect from the quantities of ammonia which are found in the urine. The 24-hour ammonia nitrogen in the urine is usually between 0.3 and 0.6 gram in the case of normal persons, and in diabetic acidosis this ammonia may be increased as much as ten times, or even more. Corresponding to such variations in the ammonia excretion there ought to be unmistakable variations in the ammonia content of the blood filtrates, even though anything approaching a constant coefficient is not to be expected.

Because of the extraordinary difficulties involved in the determination of ammonia in blood most of the literature on the subject is taken up with discussion of the necessary analytical procedures, and after more than 20 years of such effort there is still no generally accepted method available. Obscure points, such as the ammonia content of every brand of potassium oxalate and the practical impossibility of removing that impurity by recrystallizations, the persistent presence of ammonia in every brand of ethyl and methyl alcohol, the formation of ammonia in the blood on standing, or on applying heat—all have contributed to the publication of ammonia values for blood which differ by several hundred per cent. The true values for blood remain, therefore, uncertain; and the uncertainty is increased by the fact that the blood of man yields substantially the same values as the blood of herbivorous or carnivorous animals, and substantially the same whether the blood comes from normal persons or from those eliminating excessive quantities of ammonia with the urine. The expected variations in the blood, corresponding to known variations in the elimination so far have not been found.

Nash and S. R. Benedict (13) have lately propounded an interesting explanation of the origin of the urinary ammonia which would account satisfactorily for all the observed facts. According to these authors the ammonia which finds its way into the urine is produced by the kidneys. The ammonia production might thus be pictured as another phase of the regulatory mechanism in that organ whereby the needed basic materials are reserved, partly by secretion of an acid urine and partly by this localized production of ammonia. Under these conditions the amounts of ammonia in the blood have, of course, practically nothing to do with the quantities excreted.

The explanation offered by Nash and Benedict is not merely a theory. It is supported by analytical data which show that the ammonia of the renal vein contains more ammonia than other blood, either venous or arterial. Opinions will necessarily differ as to the supporting value of the experimental data. The extra ammonia in the renal vein represents an over-production or a lack of perfect excretion of the ammonia, and is, of course, not at all an accurate index to the total ammonia formation in the kidneys—a point clearly recognized by the authors.

Nash and Benedict's paper gives an excellent review of the preceding literature. The one paper which has appeared later, Gad-Andersen's (14), describes another method for the determination of ammonia, but there is no reason to believe that this latest method is the best, especially as the results obtained are said to agree with those found by Henriques and Christiansen's method, which involves the use of four volumes of alcohol.

It is clear that for the present the question of the ammonia content of blood can have no bearing on any clinical problems.

*Urea.* Urea in blood as in urine is quantitatively by far the most important nitrogenous product. The normal variations of the urea nitrogen lie between 8 mgm. and 15 mgm. per 100 cc. of whole blood. The latter figure is really outside the normal, unless the subject is on a very high level of protein metabolism. In connection with upper normal values it should be pointed out that these values may persist for 2 or 3 days or longer after the protein consumption has been reduced. A low normal level is therefore not necessarily obtained, because the blood is taken before breakfast in the morning.

As a part of the total non-protein nitrogen of human blood the urea nitrogen varies under normal conditions between 35 and 55 per cent. The proportion falls most frequently between 40 and 50 per cent, but the variations are so large that it is not safe to assume,

as is frequently done, that the urea nitrogen is just about one-half of the total non-protein nitrogen. In nephritic nitrogen retentions the increase usually involves a greater increase of the urea than of the total nitrogen, and the per cent of the latter represented by urea may rise up to 70 per cent.

Since the introduction of the urease methods for the estimation of urea (15), (16) this determination has become the most popular in chemical laboratories. The determination is unfortunately by no means so dependable as many seem to think. The enzyme employed is exceedingly sensitive, is occasionally more or less completely inactivated, and yields values that are too low. The total non-protein nitrogen determination represents, therefore, a more valuable and more dependable process for the study of nitrogen retention than does the urea determination. Both normally and in nitrogen retentions the urea is more abundant in the plasma than in the corpuscles. The distribution of the different nitrogen fractions of blood is illustrated by the table given on pp. 463 and 464. These figures were recently obtained by Berglund.

The enormous quantities of urea which can accumulate before the uremic patient finally dies indicate clearly that urea is not toxic, a fact also suggested by the extraordinary urea content found normally in the blood of the shark.

The simultaneous determination of urea in blood and in urine has received much attention in recent years as a means for determining the excretory efficiency of the kidneys. This line of investigation originated in France (17), and has been further elaborated by McLean (18). Opinions differ as to the value of such studies. The fundamental underlying assumption that the excretory power of the kidneys may be expressed in the form of a dependable constant is none too well established, however alluring it may appear to those who like to express metabolism processes in terms of mathematical formulas. The idea of the existence of such a constant certainly breaks down when it is extended so as to account for the rate of excretion of all waste products.

*Uric acid.* Of all the known definite constituents of the non-protein nitrogen there is none which has received so much attention and which is so interesting alike from the standpoint of normal and of abnormal metabolism as uric acid. It is difficult to appraise with any degree of certainty the work upon which the early findings of uric acid in blood are based. Garrod (19) cites figures for uric acid in human blood

which, to be sure, seem not unreasonable. No one probably would now be able to confirm those figures by means of Garrod's method, yet Professor Lehmann in his well-known textbook (1851) states that he could for the most part confirm Garrod's observations—only (as he states) he first happened to do so by finding uric acid in the blood of carnivorous animals (dogs) (20). Yet 60 years after Garrod, Gudzent (21) made a notable contribution by merely showing that positive murexide reactions could be obtained on dialyzates from certain clinical bloods, but not from normal human blood or from the blood of animals. It is difficult to assume that Garrod in 1848 could tell more about the uric acid content of blood than Brugsch and Schittenhelm (22) could do a dozen years ago. These latter investigators devoted much time to the subject; but, like Gudzent, they had to be content with positive qualitative tests obtainable from certain kinds of human blood. Even after the extraordinarily delicate uric acid reagent of Folin and Denis had been introduced (1912) the uric acid content of blood could not always be determined with any high degree of certainty. Modern studies on the uric acid content of blood began with the introduction of that reagent.

A few remarks may be not out of place here concerning the interesting development of the colorimetric method for estimating uric acid in blood. The blood proteins from 20 cc. of blood were removed with boiling 0.01 *N* acetic acid. The filtrates were concentrated to a small volume and the uric acid was then precipitated by the best precipitant then known, the silver magnesium mixture—a uric acid precipitant first introduced by Salkowski in 1871. After removing the silver with hydrogen sulfide and boiling off the surplus  $H_2S$  the uric acid reagent was added, together with sodic carbonate, and a fine blue color suitable for quantitative comparisons was obtained.

This basic method has since been enormously simplified and improved. The most important single improvement was the introduction of potassium cyanide (23) by which the uric acid is set free and the disturbing silver is converted into complex cyanide compounds which have no disturbing effect. The next important step was the elimination of the tedious acetic acid precipitation, and accomplishing the precipitation of the uric acid by silver lactate without any preliminary concentration (24). The most remarkable step of all was taken by Benedict (25) a short time ago when he showed that it is not necessary to isolate the uric acid from the blood filtrates, and that by applying heat so much color is obtained that the blood filtrate from 0.5 cc. of blood is

adequate for a determination. (Garrod used several hundred cc. ("2 pounds") of blood for each of his "determinations.") Benedict's last simplification is based on the use of a reagent which differs from the regular reagent in that a part of the phosphoric acid has been replaced by arsenic acid. Benedict believed that the secret of his success lay in this new reagent, but it has since been found (26) that the original uric acid reagent behaves in the same way and that the secret of the remarkable simplification introduced by Benedict lies in the use of appropriate amounts of sodium cyanide as the only alkali. Unless some now unknown flaw is discovered in these latest forms of the colorimetric determination of uric acid there is scarcely place for any further improvements, except perhaps in the matter of preparing more stable standard solutions of uric acid. This last requirement is probably met by Folin's finding that uric acid formaldehyde combinations liberate their uric acid quantitatively, when diluted with water.

The results of colorimetric determinations of uric acid in blood have on the whole substantiated the views which prevailed earlier both concerning normal purine metabolism and with regard to the accumulation of uric acid in blood in nephritis and in gout. The essential advance consists, therefore, up to date, mostly in the perfect certainty as to the correctness of these concepts and in the possibility of getting definite figures for all kinds of blood, including human blood in health and in disease. The fact learned from urine analysis that in all mammals, except the anthropoid ape and man, the chief end-product of the purine metabolism is allantoin, whereas in the anthropoid ape, in man, and in the birds, it is uric acid, is verified by the fact that in man and in birds the uric acid content of the blood is higher than in the blood of all mammals so far investigated. The uric acid content of bird's blood, curiously enough, is only about twice as high as the average normal found in man and is no higher than the values frequently found in the gouty.

The following uric acid figures for the bloods of animals were obtained by Folin and Denis (by the original colorimetric method) (27):

	RABBIT	SHEEP	PIG	HORSE	MON-KEY	OX	CAT	CHICK-EN
Mgm. uric acid per 100 cc. of blood .....	0.05	0.05	0.05	0.05	0.05	0.2	0.2	4.9

The figures 0.05 must be interpreted as meaning that no uric acid could be found, rather than that the figure cited is really correct.

In normal human blood the uric acid content is subject to relatively greater variations than that of any other known nitrogenous product. The lowest figure reported by Folin and Denis is 0.7 mgm., and the lowest found by Benediet out of 50 analyses is 0.8 mgm. The maximum normal figure for the uric acid may perhaps be given as 3 mgm. per 100 cc.

For typical cases of gout Myers (28) found uric acid values ranging from 6.8 to 9.5 mgm. without any accompanying increase of any other nitrogenous waste product. These figures average materially higher than the figures previously reported by Folin and Lyman (29) or by Folin and Denis. In twelve cases of gout unaccompanied by nephritis (normal non-protein nitrogen) these last two authors found uric acid values ranging from 3.3 to 5.2 mgm. The characteristic and extraordinary feature of pure gout is that in most cases only the uric acid is increased. Whether this peculiar fact is to be interpreted in terms of a highly selective activity and correspondingly selective deterioration of the kidneys, or whether it means that uric acid is more difficult to excrete than any other waste product, a possibility suggested by the high normal levels and by the excessive variations in the uric acid content of the blood of normal persons, is by no means clear; nor is it certain that either one of these two hypotheses can furnish an adequate explanation. In nephritis leading to true uremia there is a gradual accumulation of all the nitrogenous waste products, except ammonia, and in such bloods there is no constant relationship between the increase in the uric acid and in that of the total non-protein nitrogen. But, on the whole, one can say that the uric acid seldom, if ever, shows a greater percentage increase than does the total nitrogen; that the uric acid accumulation, on the contrary, is usually less pronounced. In other words, in cases of generally diminished kidney efficiency one finds no support for the idea that the uric acid retention represents any special difficulty in the process of excretion. The statement made above is true at least for advanced cases of nitrogen retention. Whether it is also or equally true for the early stages representing moderate nitrogen retention is not certain. Myers (30), Krauss (31) and others have drawn the conclusion that the retention and accumulation of the uric acid precedes and exceeds that of the non-protein nitrogen in the early stages of kidney insufficiency.

More observations on the problems are much needed, partly because the older colorimetric technic of uric acid determinations has been none too dependable—a situation not improved by the freedom with

which little modifications have been introduced. Figures obtained by means of clinical colorimeters or by comparison with artificial standards can scarcely have much value in relation to such a problem. Values obtained on whole blood are also less convincing than those obtained on plasma.

There is one other interesting point which must be referred to in connection with the uric acid content of blood. Benedict (32) found some years ago that the uric acid content of ox blood which is normally very low (0.2 mgm.) increased several hundred per cent if the blood was allowed to stand (with preservatives) for several days. He also found that the uric acid content of blood filtrates from ox blood gave correspondingly increased uric acid figures if the filtrates were boiled with concentrated hydrochloric acid. Following these clues he was later able to isolate from ox blood filtrates a substance in pure crystalline condition which by hydrolysis is split into uric acid and a reducing sugar. Alice Rohd  Davis, working with Benedict on the problem, has obtained analyses indicating that the sugar is a pentose (ribose). This uric acid compound is located in the corpuscles of ox blood and is also found in the corpuscles of other animals, though in smaller amounts. If present at all in human blood the amount is so small that its isolation has not been accomplished with any degree of certainty. Morris (33) has lately obtained analytical data which, according to his interpretations, indicate that some form of combined uric acid may be present in some samples of human blood.

*Creatinine and creatine.* The investigation of these two substances as constituents of protein-free blood filtrates dates from the introduction of the colorimetric method for their determination (1914) (34). Earlier investigators had tried to determine creatine and creatinine as they had tried to search for every other product found in urine, and some ventured to report actual figures. Carl Voit (35), for example, found as high as 108 mgm. of creatine per 100 cc. of ox blood. All such early attempts were necessarily hopeless. Even the colorimetric method now used for the determination of creatinine and creatine has not yielded results the validity of which can not be questioned. Like the early findings for urea or for uric acid the modern creatinine and creatine values could not have been even tentatively accepted, if it had not been for the fact that creatinine and creatine were necessarily supposed to be present, since creatine is abundant in the tissues and since creatinine is one of the major nitrogenous waste products.

The earliest figures obtained by the colorimetric process were doubtless too high for the creatinine and much too high for the creatine. The figures for creatinine should be, and probably are, more nearly correct than those found for creatine, but like most figures obtained by a correct colorimetric reaction even the latest figures for blood creatinine must be interpreted as maximum figures, thus leaving open the possibility that they may later be shown to be too high. Up to the present time there is, however, no evidence available showing that creatinine figures obtained on the basis of really pure picric acid and the right amount of alkali are materially too high, at least when applied to plasma. Figures for the blood creatine are necessarily more uncertain, because of the heat which must be applied to the blood filtrate for the transformation of the creatine into creatinine. This heat was particularly disastrous when applied in the presence of picric acid, because some substance in the filtrate acted in acid solution on the picric acid so that it gave considerable color when the alkali was added. Folin and Wu thought that this effect might be due to hydrogen sulfide. (Hydrogen sulfide seems to have some reducing effect on picric acid when heated with it in the autoclave.)

Whether the plasma of normal adults contains any free creatine must, on the whole, be considered doubtful, since such individuals excrete no creatine (36), yet do excrete creatine when sufficient creatine is taken to show any demonstrable increase in the creatine content of the plasma. The true creatine present in blood is probably confined to the corpuscles, and the corpuscle creatine is probably held in these cells by the same forces which result in the maintenance of the creatine content of muscles. The significance of all this creatine has never been satisfactorily explained. Folin regards it as a (post-mortem) product set free when the cell protoplasm is killed (37). Until some other explanation of the sharp localization of creatine in the animal body has been found this explanation should be kept in mind as at least possibly correct.

The normal creatinine content of human blood may be given as 1.2 to 1.5 mgm. per 100 cc. of whole blood, according to results obtained by the method of Folin and Wu. The creatine content of such blood varies between 3.5 mgm. and 5 mgm. The creatinine content of blood is normally remarkably constant, as might have been expected in view of the fact that the endogenous production of creatinine is by far the largest source of the urinary creatinine. Because of these facts, V. C. Myers (38), with collaborators, has stressed the point that large

retentions of creatinine represent the most valuable single index in advanced cases of nephritis in which there are nitrogen retentions. Further and more detailed investigations including particularly the concentrations in the plasma are urgently needed. Myers and Killian (39) found less creatinine in the plasma than in the whole blood in cases of excessive nitrogen retention. This finding is probably erroneous, and is presumably due to some flaw in the technic employed.

The question of the distribution of the various soluble nitrogenous products of blood filtrates between the plasma and the corpuscles is one which lately has received considerable attention partly because of the remarkable findings reported by Falta (40) and his co-workers. At present it seems measurably correct to say that if the different water content of plasma and corpuscles be disregarded, then only the amino acids, creatine, and the undetermined nitrogen are more abundant in the corpuscles than in the plasma; while the nitrogenous waste products—urea, creatinine, and uric acid—are more abundant in the plasma. In the case of uric acid the difference between the figures obtained from whole blood and from plasma is so large that practically the whole of the uric acid content of the blood falls on the plasma. If it were not for the greater practical difficulties it probably would be better for analytical purposes to substitute the plasma altogether for whole blood. Serum gives substantially the same values as plasma.

The determination of creatine, whether in whole blood or in plasma, is at present of importance only in connection with experiments which may serve to elucidate the obscure metabolic origin and significance of creatine and creatinine. Clinically such determinations can not at present be said to have any value. The creatinine determination, as already pointed out, is of considerable clinical importance.

Since the above summary was written there has appeared another paper on the creatinine and creatine of blood, by Behre and S. R. Benedict (41). In this paper the opposite side is taken on nearly every point involving the occurrence and significance of creatine and creatinine. The blood, including the plasma, is said to contain only creatine, and the creatinine figures previously reported are explained as due to the inadequacy of the available analytical technic. The creatinine content is given as less than 0.05 mgm. per 100 cc. of blood. The experimental work underlying these extraordinary conclusions is complicated. Behre and Benedict advocate the retention of the "creatinine" determination in the study of nephritis, although the chromogenic material now involved is entirely unknown. The determination should, how-

ever, not be made by the method of Folin and Wu but according to a process described by the authors.

In view of the many still active investigators who in the past have made contributions to the creatine-creatinine problem one can safely predict that the findings and conclusions of Behre and Benediet will not long remain without contradiction or verification.

*Amino acids.* The amino acid nitrogen content of blood has been already referred to. The problems of normal protein metabolism have been solved in a large measure, in so far as those problems are concerned with the fluctuations of the amino acid nitrogen of the blood. In connection with the investigation of clinical problems it should be pointed out that the determination of the amino nitrogen, by itself, has very limited application. The deamination process appears to be such a fundamental process that one cannot expect to find many pathological conditions in which the amino nitrogen of the blood filtrates will vary very much from the normal. As was stated in connection with the discussion of urea, the deamination is not affected by the accumulation of this end product. Even in the most advanced cases of uremic nephritis the amino acid content of the blood is by no means increased. In the course of a very large series of amino acid determinations on a great variety of clinical bloods Bock (42) has, to be sure, found unusually high values in nephritis when the non-protein nitrogen exceeds 100 mgm. The increases found are very variable. Bock used Van Slyke's method. Berglund, working with the colorimetric method, failed to find any increase of the amino acid nitrogen in several cases of nephritis, where the non-protein nitrogen ranged from about 70 mgm. to over 300 mgm. The normal values found by Bock ranged from 6.1 to 7.9 mgm.

That it is possible for deamination abnormalities to occur must be admitted. Cystinuria furnishes a beautiful example. For the present, however, such problems can be investigated only on the basis of urine analysis—unless one should by chance encounter a case of uremia superimposed on cystinuria.

The normal constant elimination of amino acids with the urine is interesting in view of the fact that the amino acids are not waste products. Their elimination indicates that there is no threshold, no force, by which they are quantitatively retained below a certain concentration in the blood. The level in the blood equivalent to from 5.7 to 7.8 of nitrogen per 100 cc. of whole blood, may be said to be normally very constant, but unmistakable increases occur after every substantial

protein meal, and, corresponding to these small, but definite increases in the blood, there occur considerable increases in the hourly excretion. The reason why the daily loss of amino acids through the kidneys remains small is the same as the reason for the failure of the amino acids to accumulate in the blood—namely, the avidity with which they are taken up by the tissues. The tissues absorb the amino acids almost as extensively and as perfectly as they absorb the sugars, yet for the amino acids we assume no retentive mechanism analogous to the glycogen formation.

The statement made above that deamination abnormalities are rare and that one can scarcely expect to find such on the basis of amino acid determinations made on blood must not be construed as implying the complete absence of clinical cases in which the amino acid nitrogen of the blood is high. Extremely high amino nitrogen figures are already on record. In a well-studied case of yellow atrophy of the liver, Feigl and Luce (43) found almost a twenty-fold increase of the amino acid nitrogen (from 42 up to 115 mgm.). Stadie and Van Slyke (44) have also reported very high amino nitrogen values for an acute case of yellow atrophy (14 to 26 mgm.). Other similar cases have been reported. The high amino acid value found in such cases can scarcely be designated as representing metabolism disorders. The accumulation of amino acids is not due to a failure of deamination and urea formation, but rather to an excessive production of amino acids by virtue of a rapid autolysis of one large organ—the liver. The accumulation of amino acids in these cases is analogous to the accumulation of uric acid in leukemia.

Practically all of the literature on the amino acid content of blood is based on the gasometric method of Van Slyke. The new colorimetric method of Folin (45) appears to yield substantially the same sort of values, although as yet no parallel determinations have been made on blood filtrates.

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# PHYSIOLOGICAL REVIEWS

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## THE PHARMACOLOGY OF THE AUTONOMIC SYSTEM

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A review of this subject, following so closely on the appearance of Langley's masterly little volume, *The Autonomic System*, requires unusual temerity. However, since most of the material had already been gathered and since the review had been planned from a somewhat different point of view, it seemed worth while to attempt to carry through the original enterprise; namely, a survey and orientation of the more recent developments that threatened or promised to disturb the narrow, rather rigid schema into which the pharmacology of the autonomic system had tended to "set."

The striking differences in the origin and distribution of the three great classes of efferent nerve-systems and their end organs—the somatic or voluntary system, the parasympathetic and the sympathetic—are reflected in an almost equally striking manner in their peripheral reactions to certain drugs; so much so that these could be definitely classed as sympathetic (for instance, epinephrin and ergotoxin); parasympathetic (for instance, pilocarpin and atropin); and strio-muscular (for instance, guanidin and curare). The correspondence between the drugs and innervations is so far-reaching that it cannot be accidental. There must be a fundamental, specific relation between the innervation and the drug-reactions; and if so, all poisons that act in a special manner on the peripheral system should belong, definitely and sharply, to one or the other of these groups; and we should thus be able to arrange all these drugs into a simple orderly, logical and generally satisfying system.

There is, however, another side of the picture: The correspondence is impressive, but not complete. On the one hand, some drugs affect, more or less, several of the systems; and on the other hand, the most

typical drugs seem to act on the wrong system in some organs, such as the uterus and the sweat glands. These exceptions were naturally set aside as "atypical" until the main scheme could be better digested; but they have continued to accumulate in number and in impressiveness until it seems that they might submerge the whole schema, unless the schema could be sufficiently broadened to cover them. Very evidently, the divisions of the efferent systems are not separated as sharply as the schema would imply; at least, the chasm between them is not impassable. The facts may be reconciled by the assumption that the fundamental distinction goes beyond the nervous systems; that the reactions, whether to drugs or to nervous impulses, depend on the physical or chemical properties of the reacting cell; that these vary with conditions; that a certain complexion of these conditions is ordinarily associated with each of the nervous systems; but that these conditions may be shifted, naturally and artificially; and that then the reactions to nerve impulses and to drugs are also changed, separately or together.

In other words, it would appear that autonomic specificity is not an absolute, separate entity; but that it is conditioned on the ordinary, fundamental properties of muscle, of reactive tissue; in fact, of excitability in general. Conditions which affect these are also capable, potentially, of affecting autonomic specificity. This specificity is relatively fixed, because these properties are relatively fixed in a given type of tissue.

Generalizations of this sort hold out attractive prospects of satisfying the innate longing for unification; but they have little value unless they unify actual phenomena. Perhaps the chief significance of the more recent work on autonomic drugs is therefore the collection of data concerning the conditions that modify response. As yet, these data can furnish only hints, partly because they are incomplete in themselves, and partly because their interpretation must await clearer conceptions of the various properties of excitable tissues than we possess at present. There is reason to hope that each will throw light on the other; and it would not be surprising if the paradoxical effects of autonomic drugs would be important aids in unravelling the complexities of the nature of excitation.

Incomplete as are the present data, the space at the disposal of this review does not suffice to present them with any degree of detail. It can only sketch the subject in its broadest outlines, with a bit filled in here and there, by way of illustration.

I. NATURALLY TRANSPOSED SPECIFICITY. Under this heading we shall consider the instances in which drugs of pronouncedly specific autonomic character act on the wrong system, *under normal conditions*. Drugs with more ubiquitous actions—nicotin, barium, histamin, pituitrin, etc.—will only be considered incidentally. They are inherently eclectic rather than specific; and although the distinction may in the end prove to be of degree rather than of kind, they would as yet tend to obscure more than to clear the subject of perverted specificity.

It would also be desirable to disregard quantitative anomalies for the present. The various autonomic organs require quite different doses of their specific drugs, so that some may appear to be relatively refractory. The difference may be merely a matter of penetration, or it may have a deeper significance. In any case, changes of concentration may lead to reversal of the effects on the same system; or bring out effects on the complementary system. The quantitative anomalies therefore furnish a bridge between atypical actions induced by artificial conditions (dosage), and those that occur naturally with the usual dosage.

A special difficulty arises in deciding whether a reversed response (for instance, the secondary acceleration of the mammalian heart by pilocarpin) is due to a reversed action on the same system (conversion of vagus excitation to vagus paralysis); or to a transfer of the action to the complementary system (vagus excitation to accelerator excitation). It might appear that this could be easily decided by resort to nerve-stimulation or to antagonistic drugs; but the results of these methods are often as difficult to interpret as the original problem.

It is suggestive that perverted drug specificity is confined largely to organs, in which the innervation is itself unusual or confused. It is especially common when the tissue receives only one innervation; or when one innervation predominates very largely over the other; or where both systems produce the same response. For instance, in the sweat glands, the genito-urinary system; the frog's lungs; the blood vessels; and in the unpublished instance of the frog's small intestine, perverted specificity of drug is generally associated with weak contrast in the specificity of innervation.

We may conveniently arrange the instances under the innervations that are affected:

1. *Sympathetic Innervations affected by Parasympathetic Drugs.* a. *The sweat glands:* These are often quoted as the most striking instance of perverse autonomic reaction. Those of the cat's paw were described

as innervated exclusively by the sympathetic, the electric stimulation of this causing secretion; but they fail to react to epinephrin, and on the contrary respond to the parasympathetic poisons, to pilocarpin and atropin.

The work of Dieden and of Muto (1916), however throws doubt on the interpretation of the phenomena. They find that the sweat glands receive a parasympathetic as well as the sympathetic innervation; the relative importance varying in different animals. This would leave the possibility that the parasympathetic poisons act on a parasympathetic innervation. The drops of sweat that appear on stimulating the sympathetic could be due to contraction of the muscular coat of the glands, as suggested by Gaskell (p. 37); or the failure of epinephrin to produce secretion could be due to vasoconstriction.

The example of the sweat-glands is therefore not as decisive an instance of perverted specificity as is commonly supposed.

Incidentally, epinephrin has a normal augmentor effect on the sympathetically innervated skin-glands of the toad (Wastl, 1921).

*b. The uterus:* Although this has a double innervation, the parasympathetic appears to be practically negligible. Stimulation of the sympathetic (hypogastric) produces marked effects, which may be contractor or relaxor, according to the species of the animal. In cats, the response is inhibitory except during pregnancy, when it becomes contractor.

On pregnant cats, i.e., with contractor response of sympathetic stimulation, Cushny (1910) found that the parasympathetic pilocarpin produces contraction, as well as the sympathetic epinephrin. This has been abundantly confirmed, and seems to hold for all species and conditions in which hypogastric stimulation produces a contractor response.

The results as to the efficiency of the specific antagonists are conflicting. Cushny reported that the sympathetic ergotoxin antagonized the contractor effect of either drug; but that the parasympathetic atropin antagonized only the parasympathetic pilocarpin contraction, and not at all the response to epinephrin or to hypogastric stimulation. On the other hand, Dale and Laidlaw (1912) found ergotoxin ineffective against pilocarpin stimulation; and Gohara (1920) claims that atropin abolishes the contractor effect of epinephrin in rabbit's uterus.

There is also considerable uncertainty as to the transposition of inhibitory effects. In non-pregnant cats, in which hypogastric stimulation or epinephrin usually relax or inhibit the contractions, Cushny

also noted inhibitory phenomena after pilocarpin injection; but they were much less characteristic than with epinephrin. Dale and Laidlaw did not observe relaxation when the excised non-pregnant uterus was subjected to pilocarpin, although it occurred sometimes during life. This they attributed partly to increased suprarenal output, partly to action on the sympathetic ganglia, and not a peripheral autonomic affect. Gunn and Gunn (1914), however, did occasionally observe inhibitory effects on the excised uterus of rats and guinea pigs.

These conflicting data as to the exchange of antagonisms, and as to the acquisition of inhibitory sympathetic characters by pilocarpin, do not invalidate the high importance of the undoubted fact that the contractor sympathetic innervation responds identically to sympathetic and parasympathetic drugs. It is evidently not necessary that a drug should completely lose its identity when it changes from one system to the other; and one need not expect that the sympathetic affinity of pilocarpin should reach the high perfection of epinephrin.

*c. Retractor penis:* This has a typical double innervation, with definite reciprocal response; the sympathetic being constrictor, the parasympathetic inhibitory. There would thus seem to be no good reason for transposition; but Edmunds (1920) finds that pilocarpin as well as epinephrin are contractor; that the pilocarpin contraction is antagonized by atropin; and that atropin does not prevent the inhibitory response of stimulation of the parasympathetic nerve.

There is thus apparently a complete transposition of the parasympathetic drugs to the sympathetic system; one might say that the contractor affinity of pilocarpin is in this case more powerful than its parasympathetic affinity. However, the transposition is not quite complete; for the pilocarpin is not antagonized by the sympathetically depressant ergotoxin.

*d. Urinary bladder:* This has also a double innervation; the parasympathetic being contractor, the sympathetic rather complex. The drug responses apparently are essentially true to type; the anomalies that have been described being probably merely quantitative differences of susceptibility.

Edmunds and Roth (1920) find that the differences which Elliott (1907) had found between the response to epinephrin and sympathetic stimulation disappears with appropriate doses. They themselves report that the atropin does not paralyze the response of the parasympathetic electric stimulation, although it antagonizes pilocarpin and physostigmin. This, however, is found in many organs; the nerve current

seems more effective in overcoming the atropin block than are pilocarpin or physostigmin; or conversely, the dosage of atropin required to block stimulation of the parasympathetic nerves varies widely for different tissues (c.f. V. E. Henderson, 1922).

*Other genito-urinary organs*, so far as they have been studied, seem to have concordance of drug-specificity with innervation; whether this be double, as in the ureter; or predominantly sympathetic, as in the prostate (Macht, 1922).

*e. Blood vessels*: The evidence furnished by these is suggestive rather than crucial. They appear to have only a single innervation; at least, as judged by nerve stimulation, one nervous system or the other is so predominant that it appears exclusive. With most vessels this innervation is sympathetic and produces constriction. In the few situations where dilator effects have been definitely demonstrated, they are parasympathetic. However, drug reactions indicate that other vessels also have a mechanism for dilator stimulation through parasympathetic drugs, as shown most clearly for acetyl-cholin. This produces dilatation, which is antagonized by atropin (Hunt, 1918). Perhaps the parasympathetic "receptive substance" failed to establish connection with the nervous system because it is normally activated by the chemical products arising in the local metabolism.

The effects of pilocarpin are also dilator in most cases; but a few contractor reactions have been described, which would point to transposed stimulations of the sympathetic division. The data are cited by Langley (p. 37). Hildebrandt (1920) also reports that atropin antidotes epinephrin and sympathetic nerve stimulation in frog's vessels. It is not antagonistic to barium; but removes veratrin constriction (Kondo, 1919).

*f. Heart*: Pilocarpin inhibits the heart through vagus stimulation. This is followed by acceleration, especially in mammals; in frogs, it removes muscarin standstill. This reversal is commonly attributed to direct depression of the vagus, which is found less responsive to stimulation. However, epinephrin also renders the heart irresponsive to the vagus (Langley, 1901; Kuroda and Kuno, 1916). This suggests that we may be dealing with a transposition of the pilocarpin stimulation to the sympathetic accelerators, and not with a direct transformation to parasympathetic paralysis.

*g. Gastro-intestinal tract*: One of the most striking instances of presumable transposition is furnished by the small intestine of the frog, whose behavior in saline baths has recently been studied at this labora-

tory by Dr. G. B. Roth (in press). It reacts in the usual manner to barium, by contraction; and to epinephrin by relaxation. The two drugs are mutually antagonistic, a matter of considerable interest for the localization of the barium action. Pilocarpin does not contract the intestine, as it does in mammals and turtles; or at most, there is a brief, faint and doubtful shortening; but to the contrary, it produces marked relaxation and antidotes the barium contraction, just like epinephrin. The pilocarpin relaxation is not antagonized by atropin, which is itself a depressant; although much weaker than pilocarpin. Physostigmin behaves like pilocarpin. The direction of the change does not appear to be influenced by the ion ratio or H-ion concentration of the bath.

In *other parts of the frog's intestine*, the pilocarpin and physostigmin response is in an intermediate stage: Fuchner (1918) found for the *stomach*, and Schuller (1921) for the *rectum*, that these drugs produce only a feeble contractor response; and in the rectum at least, pilocarpin prevents the contractor effect of the more fixedly parasympathetic arecolin.

A converse instance of the transposition of the parasympathetic poisons is furnished by the mammalian *ileo-colic sphincter*. Kuroda (1916) found that this is contracted not only by sympathetic stimulation and epinephrin, but also by parasympathetic stimulants; and that it is relaxed by atropin.

2. *Parasympathetic Reactions provoked by Sympathetic Drugs*. This transposition is less common; which conforms to the high degree of specificity of the sympathetic type of drug, epinephrin.

a. *Lung-sacs of frogs and salamanders*. The musculature corresponds to the bronchial muscle of mammals, but it has a different physiology and innervation (frog, Carlson and Luckhardt, 1920; salamanders, Luckhardt and Carlson, 1920). The muscle possesses a strong and continuous inherent tone, which is relaxed by parasympathetic (vagus) stimulation, and is slightly augmented by sympathetic stimulation. The response to epinephrin is transposed; i.e., it produces relaxation. Atropin presents another anomaly, for it does not prevent the inhibiting response to vagus stimulation. Pilocarpin which was tried only on salamanders, acts normally, i.e., relaxes.

In snakes and turtles, innervation and the response to drugs is as in mammals; contractor to parasympathetics; relaxor to epinephrin; except that the relaxor effect of epinephrin is still quite weak (Carlson and Luckhardt, 1920; Luckhardt and Carlson, 1921).

*b. Blood vessels.* As was discussed in a previous section, these generally respond by constriction to sympathetic stimulation; and by dilatation to parasympathetic stimulants. Although the sympathetic constrictor action of epinephrin is especially pronounced in the case of blood vessels, it produces dilatation in various situations; constantly so when lower concentrations of epinephrin are employed, or when the sympathetic excitability of the tissue has been lowered. It is not improbable that these are due to a parasympathetic transposition of epinephrin; this is strongly supported by the reaction of the pulmonary artery of frogs and turtles. In these animals, the constrictor innervation is not sympathetic, but parasympathetic (vagus; paralyzed by atropin); epinephrin, however, produces contraction, i.e., parasympathetic stimulation.

In other special vascular areas the drug-responses are still confusing. Considerable data for various drugs have been collected by the recent work of Amsler and Pick (1919) (frog-perfusion experiments, splanchnic areas and legs); Adler (1921) (ditto, lung and skin vessels); and Rothlin (1921) (rings of mammalian artery).

*3. Autonomic Reactions provoked by Strio-Muscular Drugs.* There seems to be a definite relation between the drug-affinities of the autonomic and strio-muscular innervations. Probably all of the drugs that act on the receptive mechanism of the striped muscles act also on the parasympathetic or sympathetic division, and most of the parasympathetic drugs act on striped muscle. The sympathetic division does not seem to affect muscular contraction and tone, but has been credited with influencing muscular metabolism.

The curare and nicotin group acts more especially on the autonomic ganglia, so that their effects on the terminal cells are not easily studied. *Guanidin* is perhaps more strictly terminal: The excised frog-heart (Rosenow, 1921) responds to weak concentrations by increased systole (sympathetic stimulation?); to higher concentrations by diastolic arrest (parasympathetic stimulation?). Bronchial muscle (Macht and Ting, 1922) is relaxed (sympathetic stimulation or parasympathetic depression?).

*4. Strio-Muscular Reactions to Parasympathetic Drugs.* The more powerful parasympathetic stimulants, physostigmin and acetyl cholin, stimulate the "receptive" mechanism of striped muscle in a manner strictly analogous to the characteristic strio-muscular stimulants, guanidin, nicotin and barium. The phenomena are the same for all, with mere quantitative differences that tend to disappear by varying the

dosage: *a*, The lowest effective concentrations produce fibrillary twitchings; with increasing doses these pass to clonic contractions, tonus contractions, and finally into curare-like depression. *b*, With all, the action is strongest at the site of the end-plate; which might mean that the receptive mechanism is concentrated at this place; or merely that the permeability of the cell-wall at this place is more favorable to the penetration of the drugs or ions. *c*, All continue effective for some time after the division of the nerve trunks. Most and probably all become less and less effective when the nerve has completely degenerated, presumably because the receptive mechanism, and eventually the muscle fibers as a whole, deteriorate when they are no longer used. *d*, All are antagonized, though to a varying degree, by curare, atropin and especially l-scopolamin, and the local anesthetic group. Atropin is more effective against drugs than against nerve-stimulation; a difference that it frequently exhibits in strictly parasympathetic reactions, and which is again a merely quantitative question, for sufficiently large doses of atropin block the response of striped muscle to nerve stimulation (Haffner, 1918).

The data for these conclusions, but not necessarily the interpretations themselves are chiefly taken from papers of Frank and Katz (1921) (local anesthetics, nicotin, guanidin); E. Frank and Nothmann (1921) (physostigmin and scopolamin, on human); Frank and Stern (1921) (guanidin, degeneration, procain, atropin, barium); Fuehner (1920) (guanidin); Haffner (1918) (atropin); Langley (1913 and 1914) (curare and nicotin); Magnus (1908) (nicotin, curare, physostigmin); Meighan (1919) (guanidin); Riesser and Neuschloss (1921 and 1922) (acetyl cholin, atropin, curare, nicotin, local anesthetics); Schuller (1921) (local anesthetics and caffenin); Schuller and Athmer (1921) (local anesthetics and veratrin).

5. *Sympathetic Innervation of Striped Muscle.* There is practically nothing to add to the review of this subject given by Langley on pages 69-80 of his book. Briefly, sympathetic fibers have been traced to the striped muscle fibers; especially by the degeneration method (Dusser de Barenne and co-workers, 1919). It was suggested on speculative grounds that this sympathetic innervation controls the sarcoplasm, and through it the tone of the muscle, in accordance with Bottazzi's sarcoplasmic theory of tone. De Boer (1916) claimed to have demonstrated experimentally a loss of tone on division of the sympathetic supply. Dusser de Barenne (1916) and S. Cobb (1918) showed that this has essentially no effect on the tone. The sympathetic innervation was

then credited with the regulation of the metabolism of the muscle, but without direct evidence. Langley points out the difficulty of distinguishing a sympathetic innervation of the muscles from a sympathetic innervation of the blood vessels in the muscles, and does not consider the evidence quite conclusive; but inclines to the positive side; i.e., that sympathetic nerves make direct connections with the muscle cells.

The behavior of striped muscle toward epinephrin supports the view that the sympathetic innervation does not act on muscular contraction or tone; for epinephrin does not affect these in excised muscle. On the other hand, epinephrin increases the carbon dioxide production, even in excised muscle (Martin and Armitstead, 1922), and also creatin formation (Riesser, 1916). This indicates that the sympathetic innervation may be concerned with possible functions of muscle in general metabolism; perhaps in heat-regulation.

II. TRANSPOSITION OF SPECIFIC ACTIONS BY ARTIFICIAL CONDITIONS. We have seen that normal specificity is not absolute; that the reactions of the parasympathetic and sympathetic drugs are sometimes transposed, sometimes intermingled. It is therefore evident that innervation, although the most important condition, is not the *only* condition concerned in the specificity of the autonomic drugs. These conditions could be reduced in principle to two, sensibility and dosage. Each of these in turn may be changed by a large variety of factors, of different relative importance.

Sensibility and dosage determine, in final analysis, the degree of normal action; reversion of normal action from stimulation to depression or conversely; and transposition to the other system. These are all reducible to the same principles. It will facilitate the exposition if we employ the following terms as indicated:

*Reversal of function:* Response to the organ in opposite directions (for instance, change of contraction to relaxation). This may be the result either of transposition or of transformation of action:

*Transposition of action:* From the normal to another system (for instance, an epinephrin effect on the parasympathetic innervation).

*Transformation of action:* The action being on the normal system, but in the opposite direction (for instance, a nicotin stimulation changing to a paralysis).

*Sensitization and desensitization* will be used for increased, respectively diminished, response of either normal or the transformed or the transposed actions.

Further discussion will be more profitable in connection with actual examples:

1. *Action transposed by Intensity of Electric Stimulation:* The claw of the crayfish is moved by a stronger adductor and a weaker abductor muscle. Either muscle can be placed in tone by the removal of the other. When the muscle, with its contained nerves, is then stimulated, the response varies according to the strength of the stimulus and the muscle:

Weak stimulation relaxes the adductor and contracts the abductor.

Strong stimulation contracts the adductor and relaxes the abductor.

Each muscle has a separate contractor and inhibitor innervation (the literature is cited by Gaskell, <sup>1</sup> p. 74-76). It is clear, therefore, that merely increasing the strength of the stimulus transposes it from the relaxor to the contractor innervation in the case of the adductor; and from the contractor to the relaxor innervation for the abductor.

The central nervous system furnishes numerous instances of transposition of action according to the strength of the afferent impulses, which may be more or less analogous; but it is advisable to confine ourselves for the present to peripheral reactions.

2. *Paradoxical Cardiac Effects of Autonomic Poisons.* In the excised frog heart, fed by the Straub cannula, the simultaneous action of sympathetic and parasympathetic stimulants does not result in neutralization of their effects, as might be expected; but in transposition of the stimulant action of one of the poisons to the other system. The Vienna school (Pick, 1920) therefore assumes that both groups of drugs may stimulate either of the autonomic systems, i.e., that they are really "amphotropic." They act, however, with different intensity; so that the stimulation of the complementary innervation becomes manifest only when its excitability is abnormally raised, or if the excitability of the usual innervation is abnormally depressed.

a. When under the moderate influence of parasympathetic stimulants (acetyl-cholin, etc.), the heart responds to epinephrin, not by the usual sympathetic augmentory response, but by increase of the parasympathetic diastolic, inhibitory action. This inhibitory effect is removed by atropin, so that it appears due to parasympathetic stimulation (Kolm and Pick, 1920). Analogous transposition is shown by the blood vessels and intestines.

Pick believes that the production by epinephrin of cardiac fibrillation and dilatation in early chloroform anesthesia is due to an analogous transposition, assuming that the vagus is sensitized by small doses of chloroform.

b. Nicotin and ergotamin also produce a similar transposition of epinephrin to inhibitory action (Amsler, 1920). Amsler assumes that the ergotamin paralysis of the sympathetic reciprocally increases the sensibility of the parasympathetic, and thus transposes the epinephrin action. This would also furnish the explanation of epinephrin "reversal" in other organs.

He also explains the nicotin-epinephrin phenomenon by paralysis of the sympathetic; but Hett (1920) finds that nicotin acts on the heart as a parasympathetic stimulant; so that the transposition may be more strictly parallel to the cholin-epinephrin phenomenon.

c. Physostigmin also sensitizes the heart to the diastolic action of strophanthin to such an extent, that even maximal doses produce diastolic arrest instead of the usual systolic standstill (Froehlich and Pick, 1920). Since systolic standstill seems to be connected with sympathetic stimulation, the strophanthin diastole may be conceived as a transposition.

d. Conversely, sensitization of the sympathetic by epinephrin, or by excess of calcium transposes the parasympathetic poisons (muscarin, acetylcholin, etc.) to sympathetic stimulation; so that they produce systolic contracture instead of diastolic arrest. This sympathetic action of physostigmin is prevented by ergotoxin, and not by atropin.

e. *Abolition of ventricular vagus response by excessive doses of vagostimulants.* The mechanism of this phenomenon is not clear; but it may receive mention in this place. Large doses of apparently all vagus stimulants (aconite, muscarin, physostigmin, digitalis, acetylcholin, pituitary) render the ventricles irresponsive to vagus stimulation. The sinus and auricles still react by diastolic standstill, so that the interference appears to be with the conduction of the inhibitory impulses (Froehlich and Pick). It is therefore possible that the action does not consist in reversal to the vagus effect, but that it may involve some other mechanism of the heart.

3. *The Influence of Electrolytes on Cardiac Response.* The ions of serum and saline solutions influence profoundly all the functions of the heart, and among them their response to autonomic stimulation by drugs. These ion effects are very complicated, confusing and difficult to analyze; partly because they depend on the ratio, for instance of Ca:K, as well as on the absolute quantity of each; and partly because different levels of the heart react quite differently, and sometimes in opposite directions. The practical importance of this latter fact is not yet sufficiently widely realized; for it determines the gradient of irritability, of

tone and of rhythm production; so that the "pace-maker" may be shifted at will by changes in the ion ratio (Sakai, 1914; Daly and Clark, 1921; Kolm and Pick, 1920). Presumably this applies also to peristaltic progression in general; for instance in the intestines. However, changes in ion-ratio sufficient to influence autonomic reactions are not readily produced in intact animals (Schafer, 1915).

A detailed discussion to the ion-effects would be beyond the scope of this review. The more recent data can be found in the papers of Daly and Clark, Kolm and Pick and Carter and Andrus (1921).

a. In general, changes in the potassium and calcium content act in opposite directions:

*Increase of the  $\frac{K}{Ca}$  ratio (i.e., Calcium deficiency or potassium excess)*

produces the typical phenomena of parasympathetic (vagus) stimulation, culminating in diastolic standstill (e.g., Burridge, 1912).

*Increase of the  $\frac{Ca}{K}$  ratio (i.e., Calcium excess or potassium deficiency)*

produces the phenomena of sympathetic (accelerator) stimulation, culminating in systolic standstill (Kolm and Pick, 1920).

In brief then, *calcium* seems to sensitize the sympathetic or desensitize the parasympathetic, and *potassium* seems to sensitize the parasympathetic or to desensitize the sympathetic mechanism.

(These simplified statements hold only for solutions that contain Ca, K and Na ions. The phenomena are modified if one of the constituents is entirely absent; for instance, entire absence of Ca abolishes the response to vagus stimulation (O. Loewi, 1917).)

b. When the ion-changes are combined with the other autonomic stimulations or drugs, they reinforce or transpose the effects, in the same direction as if the ordinary specific autonomic poisons were combined:

*Increase of the  $\frac{K}{Ca}$  ratio (Calcium deficiency or potassium excess),*

corresponds to sensitization of the parasympathetic (vagus) and enhances the response of the parasympathetic stimulants (muscarin, cholin, etc.) and transposes the action of sympathetic stimulants, so that the epinephrin now produces a diastolic effect (Burridge, 1912), which is antagonized by atropin, but not by ergotoxin (Kolm and Pick, 1920).

Increase of the  $\frac{\text{Ca}}{\text{K}}$  ratio (*Calcium excess or potassium deficiency*) corresponds to sensitization of the sympathetic (accelerator) and therefore enhances the response to epinephrin, and transposes the action of parasympathetic stimulants, so that acetyl-cholin produces systolic instead of diastolic standstill. The action is antagonized by ergotoxin, not by atropin (Kolm and Pick).

Increase of the  $\frac{\text{Ca}}{\text{K}}$  ratio also sensitizes to systolic standstill by *strophanthin*, whilst increase of  $\frac{\text{K}}{\text{Ca}}$  causes *strophanthin* to produce diastolic standstill (Loewi, 1915).

c. Sensitization of the sympathetic or desensitization of the parasympathetic is also produced by non-electrolytes; by lecithin and soaps; by serum; by increase of alkalinity; and by seasonal changes:

The presence of *non-electrolytes* (urea, sugar) renders the heart more resistant to calcium deficiency; which Loewi (1921) interprets as sensitization to the smaller quantities of calcium that remain. The non-electrolytes also sensitize to *strophanthin*; they may therefore be classed with the sensitizers of sympathetic action.

Treatment of the heart with *lecithin* and *soap* renders it more resistant to cholin and pilocarpin, i.e., diminishes parasympathetic stimulation and would therefore sensitize to sympathetic stimulation (Loewi, 1921).

*Blood serum* antagonizes parasympathetic stimulation (muscarin), and increases the parasympathetic depression of atropin. This is due partly to its calcium, partly to the ether-soluble extractives (Kirste, 1921).

Increase of *alkalinity*, within physiological limits (to  $\text{H}_p$ -7.8) increases the tonus (Andrus, 1919); i.e., acts in the sympathetic direction.

The *Aestival vagus insusceptibility* of frogs is not due to changes in the nerves, but to desensitization; for the inhibitory response can be restored by drugs that stimulate the vagus or that depress the sympathetic (Cori, 1921).

*Radioactive substances*: These seem to act in the direction of parasympathetic sensitization; for they seem to have the effect of radio-equivalent quantities of potassium (Zwaardemaker, 1918). Zwaardemaker suggests that the radio-energy liberates potassium (and presumably other ions) from their combinations in the protoplasm.

4. *Paradoxic Vascular Reactions, and Influence of Electrolytes:* The blood vessels exhibit the same transposition of specific autonomic drugs by sensitization of the opposing system or desensitization of the normal system, as was described under the heart; either through specific autonomic drugs, or through changes of the ions in the salt mixture, or through other means.

a. *Dilator action of epinephrin.* The ordinary constrictor action of epinephrin is identified with sympathetic stimulation. Under certain conditions this is replaced by a dilator reaction, which is presumably dependent on parasympathetic affinities; this becomes manifest especially whenever the sensitivity of the sympathetic innervation is lowered; as by degenerative nerve-section (Pearce, 1913; Engelock, 1915); by prolonged sojourn of the tissue outside of the body (Sollmann, 1905); or by very prolonged perfusion with epinephrin itself (Ogawa, 1912; Bauer and Froehlich, 1918); and by autonomic drugs and ions that depress sympathetic or increase parasympathetic reactivity.

Very small doses of epinephrin are also credited with a dilator action; it is not known whether this depends on the same mechanism.

The normal dilator response of certain vessels (studied by Rothlin, 1921) may be due to the normal preponderance of parasympathetic irritability.

b. *Sensitization by specific autonomic drugs.* The transpositions through the presence of other specific autonomic poisons are identical with those described in section II, no. 2, for the heart (Kolm and Pick, 1920).

Spleen extract also sensitizes to the pressor (sympathetic) response of epinephrin; and antagonizes the depressor (parasympathetic?) response (Collip, 1920).

c. *Relation to electrolytes.* Calcium appears to act in the opposite direction in vessels, and in the heart. Potassium and hydrogen and hydroxyl ions act on the whole in the same direction in both; (Schmidt, 1921, frog perfusion):

*Calcium excess* acts as a parasympathetic stimulant to the vessels, and as a sympathetic stimulant in the heart. The vessels are dilated, and if the constrictor response to sympathetic nerve stimulation and to epinephrin is diminished, they may even respond by dilatation.

*Calcium deficiency* acts as a sympathetic stimulant on the vessels, and as a parasympathetic stimulant on the heart. The vessels are constricted, and the constrictor response to epinephrin is enhanced.

*Potassium excess* acts as sympathetic stimulant to the vessels; as a parasympathetic stimulant on the heart. The vessels are constricted. However, the effect on epinephrin is abnormal; the constrictor effect is diminished, or may even be replaced by dilatation.

*Potassium deficiency* also acts as a sympathetic stimulant, to the vessels, as well as to the heart. The vessels are constricted and react more effectively to the constrictor action of epinephrin.

*Alkalinity increase*: Moderate increase acts in the direction of sympathetic stimulation for the vessels, as for the heart; i.e., perfused vessels constrict. This is not antagonized by ergotoxin. Epinephrin is effective (Heymann, 1921). Moderate alkalinity also promotes the contractions and tone of excised arteries (Opitz, 1920).

Large excess of alkalinity relaxes (Heymann).

*Diminished alkalinity and increased acidity*: Diminished alkalinity also constricts the perfused vessels (Pearce, 1913). The mechanism is not clear; the constriction is prevented by ergotoxin, so that it might be considered as sympathetic; but the constrictor response to epinephrin and to nerve stimulation is abolished or replaced by a dilator response (Heymann; Schmidt).

A larger excess of acid dilates the vessels; the dilator concentration coinciding with the H ion concentration that stimulates the respiratory center (Adler, 1921).

Transposition of action by sensitization and desensitization applies also to the other organs on which it has been studied; for instance, the intestines (Kolm and Pick). It is not necessary to occupy space in their discussion further than to point out that many details remain to be elaborated.

III. PERIPHERAL POINT OF ATTACK OF THE AUTONOMIC DRUGS. The energy and ingenuity that have been spent on attempts to localize the site of the action of autonomic drugs has not yet borne full fruition. Indeed, the subject has been full of disappointments—or of interest, according to the point of view. Again and again, conclusions that appeared securely established had to be revised. Methods of experimentation and deduction that appeared conclusive were found, on further examination, to be inadequate and misleading. Differences that appeared fundamental turned out to be only quantitative, subject to exceptions, dependent on conditions. Magnus in 1908 destroyed confidence in localization by antagonists, but considered anatomic methods of isolation as conclusive; it has since been shown that these may also lead to false conclusions, by being incomplete, or by injury to

other tissues, or by indirect reactions through tone, reciprocal innervation, etc. Even degeneration of nerve-endings has led to deceptive conclusions.

This experience may seem discouraging as to theories; but it is inspiring to the search for facts; for the destruction of the theories has been brought about by, and led to, the accumulation of data. The theories have therefore served their normal useful fertilizing function.

To proceed with the consideration of the points of attack: Autonomic drugs could conceivably stimulate or depress either of the two systems by acting on four principal mechanisms:

1, On the ganglionic elements; 2, on the nerve-endings; 3, on the receptive mechanism or excitability of the terminal cell; and 4, on the responsive mechanism, the contractility or secretory activity.

*1. The Ganglionic Elements.* The ganglia of invertebrates are susceptible to the whole series of autonomic drugs; for instance, the ganglion-chains of the marine worm, sipunculus (Magnus, 1903); or the cardiac ganglia of the king-crab (Carlson, 1906). With vertebrates, the autonomic ganglia react definitely to the nicotin group, as shown by the fundamental work of Langley; but wherever they can be separated with sufficient sharpness, they do not seem to be very susceptible to the other sympathetic and parasympathetic poisons. It is more difficult to confirm this conclusion absolutely in situations where they are inseparably intermingled with the terminal elements, as in the heart and in the intestines. The analogy of the limulus heart lent color to the suggestion that the ganglion cells might be the seat of the rhythmic activity and of the actions of drugs upon it. The analogy, however, is not binding, and the "neurogenic theory" has almost hopelessly lost ground for the vertebrate heart (Eyster and Meek, 1921). As concerns the autonomic drugs these act on the ganglion-free apex essentially as on the whole heart (S. Loewe, 1918; Sasaki, 1921).

The evidence for the importance of the intestinal ganglion plexus to the autonomic reactions rested essentially on the experiments of Magnus (1904) that seemed to show that the rhythmic contractions of the intestines, and the effect of drugs upon them, depended on the integrity of the Auerbach plexus. Later work, however, showed that the absence of rhythm was due merely to traumatic injury; Gunn and Underhill (1922), showed that plexus-free preparations are rhythmic; and that the rhythm can be evoked for days after the excision, whilst the ganglion cells are assumed to die quite promptly. This has been abundantly confirmed (for instance, Alvarez and Mahoney, 1922); and holds true

also for the peristaltic movements of the excised intestine, as shown by the method of Trendelenburg (1917). The results of these indicate that origin and direction of the peristaltic wave depend on a gradient of tonus and excitability, inherent in the muscle; which in turn depends presumably on adjustment to the optimum ion ration. The effects of drugs in revived intestine also agree essentially with those in intestine in which the ganglia are alive.

Carlson and his co-workers find another argument for the independent activity of the Auerbach ganglia in the capricious response of the cardiac region of the stomach to nerve stimulation: either vagus or sympathetic may produce either contraction or inhibition (Carlson, Boyd and Pearcey, 1922). From this they argue that these nerves do not act directly on the muscle, but that they are largely afferent nerves to peripheral reflex center, i.e., to the ganglion plexus. The deduction does not appear conclusive. It is conceivable that each nerve might carry both augmentor and inhibitory fibers directly to the muscles (as Tashiro (1920) assumes for the intestinal sympathetic), or end result of the same stimulation on a given muscle fiber may be modified by the state, especially the tone, of that fiber. On the whole the facts seem in harmony with Gaskell's view that the ganglion cells of the autonomic system are connector elements, analogous to the pyramidal cells of the motor system, but transplanted to the periphery. However, the final conclusion must be left to the future.

2. *Nerve-Fibrils and Endings.* Nerve-fibers, where they can be reached separately, are practically unaffected by most of the specific autonomic poisons. It is therefore improbable that the finer terminal fibrils, which seem to be mere continuations of the axis-cylinders, should be the site of the specific actions. They differ from the larger fibers in permeability, it is true; but this would cause only quantitative differences, as is shown by the local anesthetics.

Direct evidence against the nerve fibrils is furnished by the persistence of the responses to autonomic drugs in excised organs after the excitability of the nerve fibers has been lost.

The results after degeneration of the nerve fibrils and endings, following division of the nerve trunk, speak in the same direction. The effects of a number of autonomic drugs, notably epinephrin, can still be evoked after complete degeneration of the nerve. This localizes their action definitely beyond the termini that are in nutritive connection with the nerve.

The interpretation of the converse phenomenon, disappearance of the drug response as the result of degeneration, is not so decisive. The effects of pilocarpin, for instance, can be evoked like those of epinephrin, after degeneration, i.e., when stimulation of the nerve trunk is ineffective. After a time however (6 weeks for sweat, Anderson and Langley, 1904) the response becomes less efficient although the muscle still responds to electric stimulation. This probably means that in the end the muscle cell also deteriorates, "atrophies," when it is not functionally used; and that this deterioration abolishes its excitability to pilocarpin before it abolishes the contractor response to direct electric stimulation.

The response to physostigmin disappears in general earlier than the response to pilocarpin: For the eye, within a few days (Anderson, 1905); but for the fibrillary contraction of striped muscle only between the 18th and 27th day (Magnus, 1908). The graduation suggests that the same mechanism is involved as with pilocarpin, i.e., gradual degeneration of the muscle.

It is rather unexpected that the excitability to physostigmin should disappear before the excitability to pilocarpin, which is a less powerful stimulant for innervated muscle, and the less powerful antagonist of atropin; but that does not prove that physostigmin acts on the nerve endings. It may be, for instance, that the physostigmin requires a special configuration of the receptive mechanism that degenerates relatively more rapidly; or it may be that pilocarpin actually excites the receptive mechanism, whilst physostigmin acts more as a sensitizer to other excitations, which would be excluded as degeneration proceeds. In favor of this explanation is the fact that the smaller doses of physostigmin only lower the threshold of muscle (normal) to nerve stimulation; that somewhat larger doses cause fibrillary twitching only after electric stimulation, and that spontaneous twitchings require a still higher dosage (Langley and Kato, 1915).

It may therefore be concluded that there is no conclusive evidence that any of the specific autonomic poisons act on the "nerve-endings" that degenerate after section of the nerves; and that the evidence of excised organs is distinctly against this mechanism.

*3. The Receptive Mechanism.* The fact that epinephrin contracts some smooth muscles, and relaxes others of apparently identical structure and which respond alike to direct electric stimulation, implies that it does not act directly on the contractile muscle substance. The striking correspondence with the response to sympathetic nerve stimulation implies that it acts on a nervous mechanism, but the persistence of the

response after nerve-degeneration proves that it does not act upon the nerves proper. It therefore became necessary to assume the existence of some intermediate mechanism, related to the innervation, but not trophically dependent upon it, on which the epinephrin and perhaps the nerve current must act. Epinephrin does not stand alone in this respect; probably all specific autonomic poisons act through the mediation of a recipient mechanism; and even some poisons that were formerly believed to affect the contractile substance directly.

The intermediate mechanism may be conceived in several ways: *a*, as a morphologic structure; *b*, as a chemical receptive substance or side-chain; *c*, as a physical system. The available facts do not permit a decision between these; indeed, the theories themselves lack so much in detail that a decisive attitude toward them would not be justified. Consequently, there is an advantage in employing a non-committal term, such as "*receptive mechanism*" for the entire concept; and to restrict the more specific terms, "*myo-neural junction*," "*receptive substance*," and "*synapse*," to the special theories that they represent.

These theories should be examined in the light of the characteristic phenomena of the autonomic system: the fact that the autonomic cells are usually innervated by two systems of nerves; that either of these may be augmentory or inhibitory in different situations; that autonomic drugs act specifically on one or the other of these systems, in one direction or the other; and that these actions may be transposed or transformed. Any theory of the receptive mechanism must be compatible with these phenomena, and if possible seek to explain them. This task may not prove as formidable as it sounds.

*a. Morphologic structure intermediate between nerve and muscle; the "myo-neural junction" of Brodie.* This assumes that the nerves are continued into the muscle cell, and there become modified in such a way that they do not degenerate on section of the nerve trunk. This attempts only to account for the persistence of the autonomic drug-response after nerve-degeneration; it leaves untouched all the other phenomena we have indicated and is difficult to reconcile with some of these, for instance, the transposition of action. The direct evidence for it is slender and dubious; and even if non-degenerating endings exist, this need not mean that they are the site of the action of the drugs; and if they are, this could be made a part of either of the other theories.

The direct evidence for the intermediate structure consists essentially of the "*endplates*" of striped muscle: these are definite structures, which might possess modified nervous functions; and the nerve fibrils

themselves apparently lie within the sarcolemma, in close apposition to the nuclei, which might modify their nutrition. However, Langley (p. 49) cites Boeke that the intracellular network does degenerate after nerve section, which speaks against rather than for the myo-neural theory; and the nucleo-sarcoplasmic part of the sole-plate cannot be invoked, since nothing of the sort is present in the cells of the autonomic system proper.

*b. Chemical receptive substance.* Langley assumes that the autonomic drugs combine chemically with constituents of the cell—the receptive substances, or rather perhaps labile side-chain receptors of the molecule of the general cell substance. He considers that two classes of these receptors are necessary, one giving rise to contraction, the other to inhibition; the response to sympathetic or parasympathetic stimulation depending on the relative amount of contractor and inhibitor receptive substance “connected with them in the cells.”

The two receptive substances could be made to account for the response to nerve impulses, but it seems difficult to apply them to their original object, the response to drugs. It is not clear, for instance, on what epinephrin is supposed to act. If simply on the receptor substance, then why should it have any relation to the sympathetic nerve supply? If it acts on the receptive substance only if this is connected with the sympathetic nerve supply, then the receptor theory loses its chemical character and becomes essentially a theory of specific difference in the nerve itself—a supposition that Langley himself rejects. The only alternative seems to be to assume four receptive substances—sympathetic contractor, sympathetic inhibitor, parasympathetic inhibitor, and parasympathetic contractor; and by buttressing this with a sufficient number of other subsidiary assumptions, explanations could probably be devised for all the autonomic phenomena; but one could not have much confidence in so elaborate a superstructure built on such slender foundation. It is safer to return to the general conclusion that the action of autonomic poisons *may* be chemical, and *may* be exerted on the more labile portion of the general molecule of the cells; there is really no evidence that even this is really the case.

*c. The physical system of the cell as its receptive mechanism.* The Nernst theory of stimulation, i.e., changes in the electrical potential of the cell, can be made the basis of interesting explanations for the receptive mechanism and its phenomena:

The double innervation with usually contrary response could be explained by the termination of the nerves at regions of different poten-

tial in the cell, which we shall call A and B. The contrasting regions could be nuclear and contra-nuclear; central and polar; membrane and sarcoplasm; exterior and interior—it is not necessary to settle this for the present. For the sake of illustration, we shall assume the nuclear and contranuclear regions, as these are undoubtedly at different potential. As the nerves develop in the embryo and grow out to make connections with the muscle or gland cells, they would naturally be directed to one region or the other of the cell, by this polar distribution of energy, or by other causes. It may be imagined that this direction would differ for the two systems of nerves, if these present different chemical or energy conditions—due perhaps to their different distance from their nutritive ganglion cell. The parasympathetic innervation could thus be attracted to region A and the sympathetic to region B—the nuclear and contranuclear region; the exterior and the interior of the cells etc. The relative importance of these two regions may be conceived to vary for different types of tissue, and with conditions, so that either the sympathetic or parasympathetic innervation may be dominant. It is also possible to imagine that in the cell of some regions conditions exist that reverse the directive forces, and thus account for reversed innervations; or that the directive force is so nearly neutral that both innervations connect more or less with one region, or with both regions.

The physical schema also offers a plausible picture of the direction of the response: Let us assume that approach to a certain electric equilibrium, say to the iso-electric point of the protoplasm, causes contraction of the cell, through surface tension, imbibition, osmosis or some such process; and that departure from this point causes relaxation. Increase of potential at region A would affect the electrical equilibrium of the cell in the opposite direction from increase of potential at region B of the same cell; and thus contraction or relaxation would ensue according to whether the nerve connected with region A or with region B. This would explain the usual reciprocal character of the two innervations.

It does not follow that stimulation of region A produces the same direction of response for all cells; it need not even be always uniform for any one cell. The response would be influenced by the electrical condition of the cell; just as the iso-electric point of a protein is either approached or receded by the addition of an acid, according as the original protein was alkaline or acid. This may be the key to the phenomena of inhibition by the dominant innervation; to paradoxical

responses to nerve stimulation; to the effects of ion-ratios; to various colloid and anaphylactoid phenomena; and to the specificity of autonomic drugs.

These drugs may be conceived as acting chemically or physically on the cell in a large variety of ways; by producing precipitation or solution; changes of the ion-permeability of the cell membranes or of the protoplasm itself; changes in surface tension; changes in the size and surface charges of the colloids; changes in oxidation, reduction, cleavage or synthetic processes; direct chemical combination with the cell constituents, etc. Whatever the change, it must necessarily lead to a change of the distribution of potential within the system. The change produced by any particular drug must tend to react more upon one region than upon the other; and this results in more or less specificity. The high specificity of pilocarpin and epinephrin suggest that these act very predominantly at opposite regions, to produce the same changes of potential as nerve stimulation; but the phenomena of transposition suggest that they act somewhat throughout the cell, the dominant response depending on conditions, i.e., on "sensitization." Such conditions could be created by the simultaneous presence of both drugs; or by disturbing the energy distribution of the system by changing the ion-permeability of the cell wall by calcium or potassium, etc; or in anaphylactic sensitization perhaps by a change in the size of the colloid aggregates.

4. *The Responsive Mechanism*: The sharp separation of this from the receptive mechanism is of doubtful validity: the functions of excitability and contractility or secretion are so closely related that it is impossible to imagine a muscle that is excitable but does not contract; or one that contracts but it not excitable. The separation can only be made in a restricted sense; i.e., a muscle may be inexcitable for some particular kind of stimulation, but respond to others. In practice, the idea of receptive mechanism has been restricted to the transfer of nervous excitation and the excitability to specifically autonomic drugs; i.e., stimulations that may be conceived to act more especially on the contrasting A and B regions. Drugs like barium, caffeine, the nitrites, the isoquinolin group, etc., seem to have less specific relation to these regions. They could be conceived as acting more generally throughout the cell; but it is again unprofitable to go far into details at present. It is difficult to imagine how they could leave the energy-distribution quite unchanged. Indirectly, at least, they must affect the irritability to all forms of stimuli.

The general physiological conditions of the cell must similarly influence its specific irritability, at least quantitatively. This is illustrated by the influence of tone and rhythmic activity on the response to autonomic poisons.

Space does not permit the further development of this subject, nor of many other incidental problems that are highly interesting, but which have less direct application to the basic phenomena that characterize the phenomena of autonomic drug response. It is a temptation to cite these at least in the bibliography; but even this would extend the review far beyond its allotted space. The following titles are therefore, confined to the material that is directly quoted in the review.

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## THE LEUKOCYTES

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To Hewson we owe the discovery of the leukocytes, the early recognition of their importance in pathological processes we owe to Virchow, and to Ehrlich we are indebted for the development of a technique which made possible their differentiation. Ehrlich's classification of leukocytes, based on morphology and specific tinctorial reactions of protoplasmic granules, forms the base-line for almost a half-century of investigation of the varied hematological problems thus opened up. There has grown up in this period a literature of such tremendous volume that its review approaches the impossible and would in fact be undesirable, as, in the course of time, there has developed a certain stabilization of knowledge of the subject which renders many earlier discussions and opinions obsolete. My purpose therefore in this paper is rather to summarize or restate present-day opinions concerning leukocyte biology.

Any discussion of leukocytes must begin with a classification of the forms found in the circulating blood of the adult animal and the classification of Ehrlich is the one generally accepted, with, however, various reservations as to interpretation of forms and their relations. One must recognize three major groups of cells: the granular group of Ehrlich (the granulocytes), the lymphocytes, and the large mononuclear transitional group (the monocytes of Naegeli). The last two groups form the non-granular cells of the Ehrlich classification. That this grouping is valid and represents in all probability a fundamental variation in function of the different types of cells is clearly indicated by the fact that similar types of cells are found, not only throughout the vertebrate series of animals, but also quite generally throughout the invertebrates (Kollmann).

However, the claim of the Ehrlich school that the particular chemical reaction of the characteristic granules of the granular group of cells is necessarily a criterion of specific function has been challenged and possibly justly. Weidenreich in opposition to the Ehrlich view emphasizes the well-known fact that, while in man the granulation of the dominant polymorphonuclear cell has a neutrophilic reaction,

in the rabbit the morphologically and functionally equivalent cell has an eosinophilic (pseudo-eosinophilic) reaction, in the guinea pig amphophilic, and in the rat and mouse granules are undemonstrable by use of the ordinary strains. It is further recognized that within a single species of animal the reaction of one type of granule may change in the course of its development. For example, in the guinea pig the granules in the developing eosinophilic myelocyte are basophilic in reaction (Downey). Even in the circulating blood in man, in a case of sharp eosinophilia (60 per cent of 13,000 leukocytes), I have noted basophilic granules among the eosinophilic and even cells in which granules of obvious eosinophilic size and character were entirely basophilic in reaction. Yet it must be admitted that the granules of the mature eosinophilic cells all show definitely an eosinophilic reaction. In spite of these variations in reaction, one must admit that in the circulating blood there are three distinct types of mature granular cell, the neutrophilic, eosinophilic and basophilic and that the distinct granules must in all probability be related in some way to the different functional activity of the cells. I am not willing to accept Weidenreich's conclusion that the neutrophilic is the only granule in the leukocytes of man which is of endogenous origin and the result of protoplasmic activity.

The application of the Romanowsky type of stain to the leukocytes early revealed an error in the Ehrlich designation of the lymphocyte and large mononuclear groups of cells as non-granular, hyalin types. The lymphocyte is shown to contain a few coarse,—at times rod-shaped—granules, with a metachromatic reaction, while the large mononuclear group shows a fine azurophile granulation. That these granules may be as important functionally as those of the so-called granular group of cells is not to be doubted.

This gives us then five distinctive types of granules in five separate and distinctive types of cells in the normal circulation of man; the polymorphonuclear cell with fine neutrophile granules; the polymorphonuclear (usually bilobed) cell with coarse eosinophile granules; the cell with irregular nucleus (often polymorphic, sometimes round) with coarse basophilic granules; the small lymphocyte with a few metachromatic granules; and the large mononuclear (or transitional of Ehrlich) with a fine azurophile granulation. There is no tendency in medical literature at the present time to regard these as other than independent forms,—end-cells—with no development from one type into another. The Ehrlich term "transitional" for the large mononu-

clear cell with indented or lobed nucleus, has long been recognized as a misnomer. The cell does not become a polymorphonuclear neutrophile, as was supposed. There has been a shifting of discussion from the question of possible relationship and transition between these mature cells to the question of relationship between their ancestor-forms, and, further, of the relationship of these stem cells to the ancestor of the red blood cell series and to endothelium. As it is universally agreed that the primitive parent of all blood cells is an undifferentiated embryonic mesenchyme cell, the moot point is the question as to how early or how late the totipotentiality (as far as the blood is concerned) of this cell is lost. Does differentiation take place early in embryonic life, or does there survive in lymphoid tissue (including the red marrow) until late in adult life or in fact throughout adult life, a cell that is still totipotent? This question is the Shibboleth which has divided the camp of the hematologists. One group, following the lead of Ehrlich, accepts the answer of an early embryonic differentiation of cells, and constitutes the so-called polyphyletic school. While this school recognizes the presence of immature, undifferentiated cells in the hematopoietic tissues of the adult, yet they would assign to such cells the power to develop along but a single line predestined from the embryonic period. These cells, then, morphologically indistinguishable from each other, are potentially wide apart. The monophyletic school, on the contrary, following the fundamental studies of Pappenheim, Maximow and others, recognizes in an indifferent free amoeboid mesenchyme cell of lymphocyte character, with basophilic granule-free protoplasm, the parent stem cell of all types of blood cells—a polyvalent cell, persisting in lymphoid tissues throughout life. The line of development of this cell depends upon the type of external stimulus and not upon inherent latent tendencies.

The embryology of a fixed tissue is difficult, but how much more difficult the determination of relationship in a group of cells which are not only capable of independent motion and early wander from their place of origin, but which also are soon caught in the whirl of a circulating medium. In addition to general recognition of origin from a mesenchyme cell, there seems to be agreement that in all animals the first blood formation takes place in the yolk sac. Beyond this point one is forced to conclude that there must be variation in different orders of vertebrates or that observation is of such difficulty that personal opinion weighs heavy in the conclusion, for even in the later embryological studies there is wide divergence of opinion.

Thus Stockard in a study of the living yolk sac of a Teleost, (*Fundulus heteroclitus*) finds that four distinct types of cells, endothelium, hematoblasts and two types of chromatophores, develop in the same environment from apparently similar wandering mesenchyme cells. His conclusion is that these cells must have been differentiated before they wandered from the point of their origin else the identical external conditions should have produced differentiation along but a single line. In further experiments, in which the heart's action and the development of a circulation was prevented by alcoholic intoxication, he finds that although vessels are formed, they contain no red blood cells and there is not the slightest evidence of red blood cell formation from endothelial cells. A further early differentiation or predestination he believes is indicated by the fact that the red blood cells arise in the posterior part of the embryo, while the leukocytes arise later and from the anterior region.

In birds, on the other hand, Sabin, working with living yolk-sac membranes, finds that there is first developed from the mesenchyme an angioblast, from which are derived not only endothelial cells but also red blood cells. The latter may also arise from more mature endothelial cells. The endothelial cells, further, proliferate, giving rise to large mononuclear cells of the blood and the clasmatoocytes of the tissues. The forerunner of the granular leukocyte of the chick appears in tissues in close proximity to blood vessels and is at first not to be distinguished from a single angioblast. It is only surely distinguishable by its behavior after division. Two angioblasts cohere, leukoblasts separate. Sabin does not recognize a primitive common blood stem cell or "hematoblast" and apparently does not wish to identify her "angioblast" as such.

Danchakoff, however, studying embryonic chick material, concludes definitely that there does separate from the primitive mesenchyme a parent blood cell—the "hematoblast"—which develops into red blood cells if caught within a developing blood vessel, and into a leukocyte if it lies in a different environment, that of the extravascular tissues.

Thiel and Downey studying hematopoiesis in the spleen of the embryo pig, find that a "hematoblast" frees itself from the mesenchyme and develops into red blood cells chiefly in extravascular spaces. While few leukocytes are formed at any time in the spleen, they apparently arise from a similar or the same parent stem cell. Lymphocytes separate from mesenchyme cells directly without an intervening hematoblast stage.

It is difficult to reconcile such varied findings as these with the assumption of a similar type of development of blood cells in all types of vertebrates. One might perhaps better conclude it is another case of scales, feathers and hair. Gross variations in hematopoiesis and in mature cells are found in adult animal forms of the different groups. It is well known, for example, that in the adult bird, red-cell formation takes place intravascularly, while in the mammalian marrow it occurs definitely extravascularly. The loss of nucleus from the mammalian red corpuscle is another well-known deviation from the blood of lower vertebrate forms. The variation in type of the special granules of the polymorphonuclear leukocyte, seen among the members of the mammalian group, has already been referred to.

In spite of the lack of desired proof from embryological studies, there appears to have been a growing tendency to accept the unitarian or monophyletic doctrine for the origin of the blood cells, even in adult life. The polyphyletic doctrine, allowing in the adult only homoplastic origin of blood cells, like cell from like, seems too narrow to explain many pathological phenomena of hematopoiesis. It postulates too sharp a specificity of cells. The problem is practically the same as that with which one is confronted in the fixed connective tissue cells. One must apparently concede that the latter can not only lay down both white fibers and elastic fibers, but also, under certain environmental stimuli, can show a metaplasia and assume an osteoblastic function. The only difference between the assumptions for the two groups of cells is that in the fixed connective tissue cell the variation in function is manifested in a variety of extracellular products, while in the wandering blood cells it is indicated by an intracellular variation. It seems quite possible then that one may have to assume for the whole mesenchyme group of cells a greater plasticity or a more permanent possession of embryonic characters than is generally assumed or than is shown by epithelial structures. If the transformation of smooth muscle into the striated voluntary muscle type as claimed by Carey under certain experimental conditions is confirmed, we have further evidence of plasticity even in the more fixed tissues of the middle germ layer.

The cell for which this totipotentiality is claimed has received at the hands of hematologists a great variety of designations; the large lymphocyte, the indifferent lymphoid cell, the lymphoblast, the lymphogonien, the myeloblast, etc. It is a cell with large, pale-staining, vesicular nucleus and a scant rim of basophilic protoplasm without specific

granulation—a young-looking undifferentiated cell. Such a cell is found constantly in the bone marrow and in lymphoid tissue. That a totipotent cell of this character is present, in small numbers at least, in the normal circulating blood appears to be the simplest explanation of such phenomena as the occurrence of red marrow in metaplastic cancellous bone in the aorta, as described by the author, and the experimental production of red marrow in association with metaplastic bone in the necrotic kidney of the rabbit as described by Maximow. Yet such a circulating cell has not been positively identified unless one assumes with Maximow that any of the smaller and larger lymphocytes may grow into this indifferent lymphoid cell and that this latter may then show metaplastic development. I have been inclined to regard the smallest lymphocytes as end cells and to assign embryonic characters to the large mononuclear cell with round nucleus and very basophilic protoplasm.

In certain pathological blood pictures and, in particular, in the acute leukemias of marrow origin, the circulation is fairly flooded with primitive cells. One has in the acute myelogenous (myeloblastic) leukemia an unlimited proliferation of this primitive cell without differentiation. Accordingly, there is lacking in the circulation every element that might be derived from it. Red blood cells show a constantly diminishing number (an aplastic anemia); granular leukocytes and blood platelets are lacking. Only myeloblasts and lymphocytes represent the leukocytic content. In the chronic splenomyelogenous (myelocytic) leukemia one has an equally unlimited proliferation but with partial differentiation, and the blood stream is flooded with myelocytes, nucleated red blood cells, platelets (small and large) and even with the megalakaryocytes. By contrast again, in pernicious anemia differentiation is toward the hemoglobin-containing cells, and marrow leukocytes and platelets show diminishing numbers as the disease progresses. I am aware that the "crowding out" theory has been advanced to explain the phenomena just cited, but it seems less satisfactory. The monophyletic theory seems eminently satisfactory even though it offers some difficulties in addition to the lack of absolute proof. Danckakoff objects that indifferent cells in marrow tissue should be subject to the same stimuli and thus differentiation should proceed in the same direction in all. I doubt the validity of the premise upon which the objection rests. Evidence tends much more toward the view that physical and chemical conditions are not uniform throughout even small masses of tissue and probably not throughout even the

colloid mass which constitutes the body of a single cell. Varying distances from the source vary the intensity of normal and toxic stimuli, oxygen tension and the like.

The assumption then of the totipotential cell simplifies the consideration of hematological problems if one can avoid the complicated schemes of development and relationship propounded by certain advocates of the monophyletic theory, notably Pappenheim. From this indifferent cell there is differentiated, as I have suggested elsewhere, a sufficient amount of varied marrow and lymphoid tissue in the body to maintain a fairly constant circulating number of red and white cells. Production keeps pace with destruction, as is shown by the constant ratio manifested in the circulating blood. While there is a rather wide variation in the normal number of leukocytes and the percentage of different forms as seen in the differential count in different people, for an individual in health the number and the differential picture are fairly constant. One may still say the average normal total leukocyte count is 7500 per cu. mm. (7300, Miller's figure for 280 cases). However, the author was surprised, in making differential counts on supposedly normal students at Wisconsin University, to find the proportion of the various forms of cells quite different from that given by Ehrlich. The 70 to 72 per cent of neutrophile leukocytes of the Ehrlich data was strikingly above the average given in the series of the author's counts (54.6 per cent) in which a Romanowsky type stain was used instead of the Ehrlich triple stain. Miller's repetition of the work on a much larger scale gives an intermediate figure of 62 per cent, in counts with Wilson's stain. I am inclined to accept Miller's figure not only in view of his larger material, but also as a result of my long-continued study of the changes produced in the leukocytic formula by low grade chronic non-disabling infections, particularly of the upper respiratory tract which are particularly prevalent in the locality in which my counts were made. Latent "childhood" tuberculous infection is quite apparently the modifying agent in some of the counts given.

The occurrence of a normal individual leukocyte formula raises immediately the question of its importance. Has it physiological significance and if so what significance? In answer to the question, it would seem that at the present time we have no evidence of what might be called a purely physiological function for the leukocytes. As far as it is known, their function may be said to be pathological. At least it is protective, and less physiologically protective than that of

skin. It is rather difficult to accept this conclusion when one considers that the leukocyte is a much more primitive cell than the physiological red cell, and appears much lower in the animal scale than the latter. Yet one must recall that protection of the body from attack and invasion by alien and enemy forms is needed by the invertebrate as well as by the vertebrate animal. In man and the higher animals all the exposed surfaces, inner as well as outer, are crowded with organisms, many of which are pathogenic and capable of invading the body or harming it by toxin production. Finding evidence in certain acute infectious diseases (measles, influenza) that pyogenic complications occur commonly when the total number of polymorphonuclear neutrophile leukocytes falls below a certain level per unit volume of blood, it has seemed justifiable to conclude that their number per unit volume is normally maintained at a protective level against these same pyogenic organisms present on mucous membranes. Bunting and Huston have described in normal animals the daily emigration of billions of lymphocytes upon the mucous membranes and in particular upon that of the intestine. It would seem that they fulfil their functions there normally. As the lymphocytes are not phagocytic it is suggested they act as affixers of toxins, and thus contribute to the immunity which the intestine has to the contained organisms. Limiting the function of these leukocytes to that of protection may be simply the expression of ignorance, yet it would appear to measure up to the present knowledge we have concerning them.

For further consideration of the leukocytes it would appear best to take up each type of cell separately.

*The neutrophile leukocyte.* Neutrophile leukocytes are found in the marrow of the human fetus between the fourth and sixth month. They are also found in connective tissue elsewhere, especially about the thymus and in the liver (Browning). In the sheep, Goodall finds an occasional leukocyte in the liver in the 2 to 3 cm. embryo; in the 4 to 5 cm. embryo leukocytes are fairly numerous about the thymus; above 5 cm. there is beginning hematopoiesis in the marrow which becomes active at 10 cm. After birth, leukocyte formation in the higher animals occurs normally only in the marrow. The mature neutrophile, with its characteristic nucleus, is a development through well-recognized changes from the myelocyte with round vesicular nucleus and a neutrophilic granulation. The myelocytes arise both homoplastically by mitosis in similar granular cells, and heteroplastically by the development of granules in a primitive cell with hyalin

protoplasm. I have emphasized the fact that the marrow cells are grouped in extravascular centers, in which the cells develop from the center toward the periphery, so that the mature cell comes to lie on the outside, adjacent to venous sinuses. The mature neutrophile, which is the most actively amoeboid of the circulating cells, apparently enters the blood stream through its active efforts and under some unknown chemiotactic influence. Equally unknown is the regulatory mechanism of the supply to the circulation. A normal marrow apparently contains a considerable supply of mature leukocytes. This is seen in sections of red marrow. It is also indicated by unpublished experiments in this laboratory which show that in the rabbit so slight a stimulus as the injection of 1 cc. of normal salt solution in an ear vein will call out a total of 300,000,000 neutrophiles (pseudo-eosinophiles) in one hour. This time interval is too short for the increase in circulating cells to represent other than an increased out-pouring. It is not apparent why but a certain proportion of these cells enters the blood stream normally and the rest remain in the marrow as a reserve.

As the neutrophile enters the blood stream its nucleus usually shows three lobes with connecting chromatin bands. Apparently as a result of ageing, the lobes increase in number to 4, then to 5 and even to 6, as emphasized by Arneth, who has constructed definite formulae, indicating the degree of marrow activity. The length of life of the neutrophile within the circulation and its ultimate fate have not been absolutely determined; Weiskotten and Steensland, by argument not entirely free from criticism, have concluded from leukocyte curves obtained in benzol poisoning in the rabbit, that the neutrophile lives 3 days in the circulation.

The granules in the protoplasm of the neutrophile are held to be endogenous and the result of protoplasmic activity, even by Weidenreich, and they are the only leukocytic granule so accepted by him. That any feature of its functions is displayed while in the normal circulation we have, as stated earlier, no evidence. The part played by the cell in pathological processes, in the coagulation of blood, in the digestion of proteid by its proteolytic enzyme, active in an alkaline medium (Opie), and in the ingestion and destruction of bacteria, in particular of the so-called pyogenic group, is too well known to need more than mention. It may be said however that it is becoming more evident that the neutrophile is not an active defensive agent against all infecting organisms, but that in several acute infections, notably typhoid fever, measles and influenza, and in at least one chronic infection,

tuberculosis, there is a marrow inhibition, with a diminished number of neutrophiles in the circulation. Defense seems to lie with the lymphoid and mononuclear groups of cells. Even in pyogenic infections, the endothelial phagocyte is found to play a part almost if not quite as important as that of the neutrophile.

*The eosinophile leukocyte.* Eosinophile leukocytes are found in the fetal tissues as early and possibly earlier than the neutrophile. Brown-ing found them in the walls of the hepatic and umbilical veins in a fetus of 10 weeks and in the marrow at  $3\frac{1}{2}$  months. The eosinophile of the adult circulation is a cell of marrow parentage, and like the neutrophile is descended through a series of myelocytes which may have had either a homoplastic or a heteroplastic origin. The theory championed by Weidenreich among many others that the eosinophile granule represents not an endogenous formation but exogenous material related to hemoglobin or its dissociation products, seems so far removed, as even a plausible deduction from the easiest observations and experiments, as to be an absurdity. The whole question of the eosinophile has been recently reviewed by Downey and by Ringoen, who give abundant proof of the opposing theory, if it were needed. With the early disposal of the theory that the eosinophile granule was but a later stage of the neutrophile, and with the demonstration of the endogenous nature of the granule one may recognize in the eosinophile a cell of specific character. Whether or not all eosinophiles are of marrow origin must apparently remain a question still to be discussed and investigated. Ringoen, apparently, is convinced that eosinophiles are formed in the hemo-lymph nodes of normal sheep, as well as in the marrow. In a study of hundreds of human lymph nodes I have never seen evidence of eosinophile production in them. Emigration of eosinophiles from the blood stream into lymph nodes is of extremely common occurrence, especially in pathological conditions, in which there is a destruction of lymphocytes within a node.

The function of the eosinophile cell is problematical. It is ordinarily not phagocytic. While its number in the normal circulation is small, under stimulation of the marrow and reaction of the latter the number in the blood may be augmented more than a hundred fold, with every evidence of emigration of vast numbers from the circulation. The cell appears to share in the defense against various animal parasites. In addition it is found in the tissues, in inflammations of the skin and mucous membranes of varied etiology and in the neighborhood of tumors of great variety. Eosinophilia is one of the phenomena of

anaphylactic shock. Ringoen sees a possible chemiotactic influence in the products of autolysis of cells as suggested by Fessinger, as an explanation for the appearance of the cell under this great variety of circumstances.

*The basophile.* The basophilic cell of the blood was at first designated a "mast cell" by Ehrlich on the supposition that it was identical with the "mast cell" of the tissues. The work of Maximow, Weidenreich, Pappenheim and others has shown such marked differences between the two cells that they must be recognized as of two distinct orders with a basophilic (metachromatic) reaction to granules in their protoplasm as the only common feature. According to Maximow there is to some extent a ratio between the number of basophiles in the blood and that of the tissue mast cells. Animals with many circulating basophiles have few in the tissues and the contrary is also true. The status of the circulating basophile has apparently not been definitely agreed upon. Pappenheim, Weidenreich and others consider it not a true granulocyte but a degenerating cell, the granules representing products of degeneration. Maximow and Downey, among others, are in accord with the original Ehrlich conception that it represents a specific granular cell of bone marrow origin. They describe homoplastic and heteroplastic development of basophilic myelocytes from which the circulating cell is derived. Physiological observations seem to confirm the latter point of view which was arrived at by the authors mentioned from a study of tissue sections. Circulating basophile leukocytes are increased relatively and absolutely in at least two conditions in which one has evidence of increased marrow activity, chronic myelocytic leukemia, and polycythemia vera. They are also increased in chronic inflammations of the accessory nasal sinuses. It would seem that they must be regarded as specific cells; but we are totally in the dark as to their function.

*The large mononuclear and transitional of Ehrlich.* These largest cells of the circulation, the so-called large mononuclear leukocytes or monocytes have been the subjects of much discussion relating to their origin, to their relation to other cells, and to their function. In the circulating blood we find, normally, large cells with two types of nuclei. The one has a large round or oval nucleus and a very basophilic protoplasm and forms in the author's large series of differential counts upon human blood, but 0.2 per cent of the circulating leukocytes, upon the average. The other has a nucleus of varied shape, at times merely indented, more commonly trilobed, at times S-shaped, and

there is an abundant protoplasm somewhat basophilic and containing fine azurophilic granules with Romanowsky-type stains. This cell is more numerous than the former and constitutes approximately 6 per cent of the circulating leukocytes. It is this cell to which Ehrlich gave the name "transitional" on the mistaken theory that it represented a stage in the development of the neutrophilic leukocyte. Ehrlich was uncertain whether its origin was from the spleen or bone-marrow but considered the latter most probable. For many years this cell was considered to be the macrophage of Metchnikoff, and thus concerned in phagocytosis. Mallory and his students have spoken of it as the "endothelial leukocyte" on the supposition that it is identical with the phagocytic endothelial cells freed from the lymph sinuses and hem-vascular walls. In her recent article in these REVIEWS Sabin practically accepts this view as a result of her investigation of the growing vessels in the yolk-sac of the chick. She finds multiplication of endothelial cells which wander both into the lumen of the vessel (monocytes) and into the tissues (clasmatoocytes). In adult animals there is also shown to be multiplication of endothelial cells and their separation from the vessel wall and entrance into the circulation (Evans, McJunkin, Simpson et al). There is no doubt that under proper stimulation experimentally or under certain pathological conditions endothelial cells may be found in the circulating blood. Simpson in Evans' laboratory has shown that with the repeated injection of colloidal dyes into animals these cells occur in showers and may constitute 90 per cent of the leukocytes in the right heart, yet at the same time constitute but 0.1 per cent on the left side. Yet this is not proof, as some authors seem to contend, that endothelial macrophages and large mononuclears are identical.

The application of vital staining to the leukocytes, both intravital and on the slide (supravital method), it has been hoped would settle this question. It is perhaps unfair to depend entirely upon a so-called biological method to determine cell-relationship. Because two cells ingest or absorb the same type of dye-stuff would not seem an absolute proof of identity. It can be shown experimentally that both endothelial cells and polymorphonuclear neutrophils will engulf staphylococci. They are thus far biologically related. Likewise large mononuclears and endothelial cells will behave alike toward certain dyestuffs, differently toward others. The results and interpretation of results vary in different hands. Without pursuing the controversy at length, I think the fairest statement of what the present day point of view

must be, is the conclusion of Miriam Simpson, that while her extensive work with vital stains "points strongly to the biological affinity of these two interesting cell types, it cannot be said to show an identity or that one may be transformed into the other in the free or circulating blood stream. This evidence is in consonance with what we know about a similar lack of transformation of one type of leukocyte to another in the blood current, our information tending to bring the conviction that a cell once shed into the stream henceforth usually undergoes no significant transformation."

My own study of the leukocytes of the circulation in normal and pathological states has led to the conclusion that the large mononuclear with the round nucleus is an immature cell and is found in the normal circulation largely as an accident. In its mature form it is the transitional cell of Ehrlich. The cell of this type, found normally, is a marrow cell and is increased in marrow reactions of a proliferative character. Yet in certain pathological conditions with unusual stimulation of the lymphoid tissue all the large mononuclears found in the blood are not of this origin. The large mononuclear group becomes heterogeneous and includes many cells of lymph gland origin. This is true not only of the acute lymphatic leukemia picture, but also of the blood in such infections as smallpox and measles. Endothelial cells may also be found in the blood in these diseases.

*The lymphocyte.* It is quite generally agreed that most, if not all the lymphocytes of the circulating blood are derived from the lymphoid tissue in the body and reach the circulation through the large lymph channels. This would seem abundantly proved by the work of Rous, Carlson and Davis, and Bunting and Huston. I find, however, as late as 1908 Gulland and Goodale expressing the opinion that most if not all of these cells of the circulation have their origin in the bone marrow. Although extramedullary lymphoid tissues furnish the main source of lymphocytes, it is generally accepted that typical areas of lymphoid tissue are found in the marrow, and that this tissue may furnish some cells to the circulation.

In the normal circulating blood one finds smaller and larger lymphocytes. The larger have the same type of nucleus, but this is surrounded by more protoplasm of a less basophilic reaction, containing usually more coarse granules. It seems safe to assume that this represents but an older stage of the smaller cell. It is not to be confused with a larger type of lymphocyte with very basophilic protoplasm which appears in the circulation under intense stimulation of the lymph glands

in certain infectious diseases, notably measles and smallpox, and also in the acute type of lymphogenous leukemia. This last cell is present in normal lymphoid tissue but does not normally appear in the circulation. Certain unpublished observations of the author and Mr. Huston show that if one makes impression preparations from lymph glands of the rabbit and measures the cells, while all gradations are found, the greatest number of the cells group themselves into three major groups; the largest cells, corresponding to the lymphoblast, have a diameter of from 18 to 20  $\mu$ ; an intermediate group which varies between 10 and 12  $\mu$ ; and the groups of smallest cells between 5 and 7  $\mu$ . Mitotic figures were found in cells of the first two groups, but were not seen in the smallest cells. This suggests a development similar to that of the red cell in which the intermediate red cell of Howell stands between the megaloblast and the normoblast.

It is generally accepted that it is by mitosis in the lymphoblasts of the germinal centers of the lymph glands that the development of the lymphocyte is inaugurated. This intermediate sized cell with its capability of mitotic division and thus of a geometric progression in the multiplication of cells, has not been generally recognized. It may, however, account for the undue number of small lymphocytes apparently produced by mitosis in a few lymphoblasts.

There is still uncertainty as to the embryology of the lymphocyte and even of the post-natal origin of the cell. Some, as Danchakoff, maintain the development of the lymphocyte from a hemoctyblast, the primitive blood cell cut off from the mesenchyme. Others, as Thiel and Downey, maintain that small lymphocytes are cut off directly from the mesenchyme cells without the intervening hemoctyblast stage. Sabin's study of the lymph nodes indicates the possibility of this latter mode of development without, in her opinion, proving it. Weidenreich and Downey hold it definitely proved that in post-natal life, the reticulum of the lymph glands, the fibroblastic tissue of the omentum, and possibly also that of the loose connective tissue retain the capacity of the embryonic mesenchyme to liberate free lymphoid cells. The orthodox view holds the lymphoblast of the germinal center as the parent of the lymphocyte in the adult animal. The necessity of postulating a tissue lymphocyte has largely departed with the general recognition of amoeboid motion in the lymphoid cell, yet complete harmony of views on the origin and relation of the cell cannot be expected at the present state of our knowledge.

The work of Rous, Carlson and Davis, and others has shown that more lymphocytes enter the blood stream during 24 hours than are present in the circulation at any one time. Bunting and Huston, in searching for a solution as to what becomes of this large number of cells in normal animals, have found that they not only leave the blood stream but also the body finding their way out onto the mucous membranes and in particular onto the intestinal. Their normal function is apparently fulfilled there. This function is unknown. If it be protective against the bacterial content of the intestine, it cannot be of a phagocytic nature, as the lymphocyte is not a phagocyte. It is suggested that it may be antitoxic or, in other words, that the lymphocyte may contribute to the immunity of the intestine to its contained bacteria by affixing toxins. During the recent years the lymphocyte has gained increasing consideration as a defense cell in pathological processes. The work of Murphy and his associates has demonstrated that it is an important cell in the defense of the body in such infections as tuberculosis and against cancer. The nature of this defense, again, is unknown. Owing to its lack of phagocytic power one seems left with but the possibility that the lymphocyte acts by affixing toxins and if not destroyed by that toxin, by the production of antibodies. The normal distribution of lymphoid tissue in the body, the position of lymphoid cells in pathological tissues all speak for such a theory.

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It seems unnecessary to further condense this inadequate summary of hematological problems into a formal set of conclusions. In the present state of our knowledge, judgment passed on many of these problems would be premature and would have only the weight of opinion. It must be left to further investigations to determine in full the life history and the functions of the different leukocyte forms.

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## THE PRESENT STATUS OF THE FUNCTIONS OF THE THYROID GLAND

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We probably possess a more detailed knowledge of the embryology, anatomy, physiology, chemistry and pathology of the thyroid than of any other gland. But as regards its interrelations with other tissues,—a vastly greater and more important field,—it can truthfully be stated we are only at the beginning of any definite knowledge.

No attempt will be made to refer to or review all the literature relative to thyroid physiology that has appeared during the last decade. Excellent reviews of this type are available in an article by Vincent (1) in 1911 and in certain textbooks, notably Biedl's (2). Rather a critical summary of the present status of certain phases of our knowledge of this gland and some of the important steps involved in acquiring it as they appear to the author will be attempted. Owing to the recent great popular interest in endocrinology (one is tempted to say endocrinimology) the thyroid gland has suffered with the rest, though perhaps less than its sister glands, from loose speculation. This, however, is only because we possess a greater number of unchallengeable facts relative to the thyroid.

**ANATOMY.** *a. Ancestry and embryology.* Owing to the survival of one class of vertebrates—the Cyclostomes—it has been possible to establish the ancestry of the thyroid of all higher vertebrates as a direct metamorphosis of the endostyle organ. The endostyle organ is an elaborate ventral mid-line pharyngeal gland in Tunicates, Amphioxus and Ammocoetes (3). In Tunicates and Amphioxus it opens into the pharynx through a groove extending the full length of the organ (5). In Ammocoetes the opening into the pharynx is reduced to a large duct (4). During the metamorphosis of Ammocoetes the endostyle organ undergoes atrophy with complete loss of three of its specialized types of epithelium including the duct, and the ductless thyroid follicles of the adult are formed from one persisting type of endostyle epithelium (6). These cells sometimes retain their cilia after metamorphosis. The ductless thyroid follicles in Cyclostomes arise solely from the endostyle

organ. Studies in the embryology of the thyroid of fishes, amphibians, reptiles and birds have shown that it also arises solely from a median single ventral tubular downgrowth of the pharyngeal endoderm in or slightly anterior to the first aortic arch (8), (9). This symmetry and uniformity of development was believed to be departed from in mammals owing to the discovery by Stieda (10) of the so-called lateral thyroid anlagen. These lateral thyroid anlagen were believed to give rise to the lateral thyroid lobes and to be developed from the fourth gill pouches, or more accurately in man from the rudimentary fifth. The view that the thyroid in mammals arises from three separate anlagen persists and still appears in many textbooks. Recent studies of the fate of the so-called lateral thyroid anlagen, post-branchial bodies, or better, ultimo branchial bodies, show that they are only atrophic remnants from the fifth pair of gill pouches which, during development, may become attached to or even embedded in the lateral thyroid lobes, but take no part in the formation of thyroid tissue (11), (12). This solution of the origin of the mammalian thyroid makes it possible to interpret many of the pathological changes and developmental defects of the thyroid, and makes the origin and development of this gland uniform throughout all vertebrates (7), (13).

*b. Gross and microscopic.* Morphologically the thyroid is one of the simplest of body tissues and resembles the lung more closely than any other tissue. Indeed, there are many embryological, anatomical physiological and pathological relations between the thyroid and the lungs the study of which has added much to our present-day conception of interrelationships. The thyroid tissue is one of the most labile tissues in the body—capable of rapid overgrowth and of equally rapid involution. Its wide range or cycle of morphological changes makes it possible to detect easily morphological changes which if interpreted in terms of function are but little removed from the normal, but if compared with similar types of morphological changes in other tissues less well endowed with the capacity for growth have frequently resulted in drawing wholly unjustified conclusions regarding the alteration of function.

The thyroid has only one known cycle of cell changes and it tends to repeat this cycle in response to all stimuli involving increased functional activity.

The normal human thyroid weighs between 20 and 25 grams and does not exceed 0.35 gram per kilo of body weight (36), (14). Statistical data indicate that the thyroid is slightly larger in females per unit

of body weight. The gross outline of the thyroid is quite variable, the greatest variation occurring in the isthmus or pyramidal process (16). In the strictly normal human thyroid the isthmus is a band of tissue from 1 to 2 cm. in width and from  $\frac{1}{2}$  to 1 cm. in thickness connecting the two lateral lobes across the trachea anteriorly just below the level of the cricoid cartilage. The presence of a pyramidal process and thyroglossal stalk must be considered as due to incomplete absorption of this portion of the thyroid tract which normally takes place between the fifth and eighth week of fetal life. In severe endemic goiter districts approximately 95 per cent of human thyroids have well-formed pyramidal processes and thyroglossal stalks frequently continuous with the foramen cecum (15). Similar variations are seen in animals; thus, in congenital goiter of dogs and sheep the thyroid lobes are usually joined by an isthmus while normally in these animals the isthmus undergoes absorption before birth.

The gland is invested with an outer fascia which strips readily and exposes a slightly lobulated smooth surface formed by the inner or true capsule. This is thin and translucent. Thickened portions of this capsule (trabeculae) extend into the gland, support the blood and lymph vessels and give it a slightly lobulated appearance (34), (35). The color of the normal thyroid varies from a pale translucent amber red to a bright translucent amber red. The normal gland is of firm consistency and made up of closely packed circular or oval closed alveoli filled with a glairy honey-colored viscid globulin—the so-called colloid, which gives to the thyroid its specific characteristic, chemical as well as physical. The thyroid unit or alveolus is similar in all vertebrates (30), (31), (32). In man these alveoli vary from 0.1 to 0.5 mm. in diameter and are lined with a single layer of low cuboidal epithelium (columnar epithelium always indicates hypertrophy) (17). The older observers (Langendorf (18), Biondi (19), Hürthle (20) and others) recognized two types of gland cell, the so-called chief and colloid cells. The former are more granular while the latter contain in addition vacuole-like globules filled with a thin fluid which some have considered as a thyroid colloid, similar in many respects to that contained in the alveolar spaces (33).

In recent years a great deal of attention has been paid to the finer specialized cytoplasmic structures especially the mitochondria and the reticular material or Golgi apparatus (27), (28), (29). Cowdry (21), (22), (24) has reviewed the literature of both these subjects. Mitochondria (Altmann's granules) are present in all thyroids. They are

very rare in the fetal thyroid and increase with age (23). Their number also varies with the size of the cell. Their lipoidal nature distinguishes them from secretion granules with which they were long confused. They can be correlated with functional activity as well as with cell growth. Bensley noted that the more active the cell the more numerous were its granules. Some authors, notably Goetsch (25), have in addition attempted to establish a relation between the pharmacological activity of the cell's secretion and mitochondria, particularly in exophthalmic goiter. This view has not received much support for the reason that the very actively hyperplastic columnar cells seen in the thyroids of myxedema are equally rich in mitochondria. They seem closely related in the functional activity of the cell but unrelated to the pharmacological value of the cell's secretion. In the thyroid cell, mitochondria are most numerous in the region between the lumen and the nucleus while in ordinary gland cells like the salivary or pancreas these granules are most numerous in the region between the nucleus and the basement membrane. Bensley pointed out that this was probably due to reversed polarity. Recently Cowdry has studied the thyroid cells of the guinea pig, using the reticular material or Golgi apparatus as an indicator of polarity. He found that the reticular material of the guinea pig thyroid cell was located in the region between the nucleus and the base of the cell in about one cell out of every five hundred, i.e., reversed, and accepts Bensley's (26) explanation that it indicates the ability of the thyroid to secrete either toward the blood-stream or toward the alveolar lumen, and this reversed secretion (toward the lumen) comes into play when the thyroid secretion is being produced in excess of the body needs. Reversed polarity has been observed only in the thyroid gland and as the ancestral thyroid (endostyle organ) was an external secreting organ it suggests that the reversed polarity is a relatively recently acquired characteristic to meet a change in function.

Lymphoid tissue, represented by scattered small foci in the stroma, is normally present in the thyroid (37). Under certain conditions associated with general overgrowth or persistence of lymphoid tissue, as in status lymphaticus, Addison's disease and exophthalmic goiter, this lymphoid tissue may undergo an extraordinary hyperplasia with the development of well-formed germinal centers.

Accessory thyroid tissue other than that found along the thyroglossal tract occurs with great frequency. In dogs and cats accessory masses may be demonstrated in upwards of 90 per cent. The most frequent

sites are in the thymus gland and in the region of the arch of the aorta.

In man there are normally small groups of undeveloped thyroid cells lying in the stroma between the alveoli. These have been spoken of as cell rests and their relation to the development of thyroid adenomata was first pointed out by Wölffler (43). It is believed that the thyroid anlage is capable of and actually produces during embryonic life many more cells than are usually required for functional needs. Under ordinary conditions, as in the case of striped muscle fibers during fetal life these thyroid cell rests undergo gradual absorption beginning in intra-uterine life and continuing after birth. On the other hand, in the presence of a stimulus for increased thyroid activity, these undeveloped rests respond with growth and become the adenomata which are almost universally present in and an integral part of endemic human goiter. Thyroid adenomata are exceedingly rare in the lower animals.

These adenomata are highly variable in size, number and structure. One recognizes types of adenoma composed of closely packed small undistended alveoli—so-called fetal adenoma, and also types with well differentiated large colloid-containing alveoli and finally all gradations between these. Morphologically different types may be present in the same gland. It is believed these variations in morphology depend upon *a*, the stage of differentiation of the cell rest from which the adenoma arose; and *b*, the degree of differentiation occurring during its growth and involution. Adenomata are capable of taking up iodine from the circulation and of elaborating the iodine-containing hormone, thus differing sharply from thyroid carcinoma. The more differentiated types as regards evidence of functional activity approach that of normal thyroid while the fetal types may or may not be able to take up iodine even after the prolonged administration of iodides (44), (45).

*c. Circulation.* The thyroid has a very large blood supply variously estimated from 3.5 to 5.9 cc. per gram per minute (66), (67). Obviously in a tissue with such wide variations in functional activity correspondingly wide variations in the blood supply must be present. Control studies should be made with standardized thyroid and the thyroid happens to be the only tissue which at present can be standardized. This is accomplished by iodine administration. The thyroid arteries form rich anastomoses on the surface but none in the depths of the gland (40). Schmidt (38) and Horn (39) have described endothelial buds or "knospen" in the arterioles which may act to reduce the speed of the blood flow by a baffle-board effect. This also reduces the pulse-wave effects which in a gland with such a short and wide capillary

path would otherwise easily pass through to the veins, especially in the marked hyperplasias. The thyroid gland can be perfused readily at a pressure of 15 to 20 mm. Hg. while the kidney requires from 70 to 80 mm. Hg. pressure. The capillary network surrounding each alveolus is comparable to the capillary network surrounding the lung alveolus. The thyroid is also richly supplied with lymphatic vessels which are collected for the most part into two major channels which leave the gland with the large veins (41). The thyroid hormone can pass directly into the blood stream, as has been shown by Rogoff and Goldblatt (42). There is no evidence except the older morphological evidence that it may also be discharged into the lymphatics.

*d. Innervation.* The anatomical studies of Berkeley (46), Anderson (47), Rhinehart (48) and others have shown that the thyroid is richly supplied with nerves, all of which are believed to belong to the sympathetic system. These nerves leave the spinal cord between the second and seventh thoracic segments and pass upward to the middle and superior cervical ganglia from whence they are relayed to the thyroid *a*, directly along the blood vessels, or *b*, indirectly through the superior laryngeal and possibly the recurrent laryngeal nerve. These nerves are distributed both to the perivascular tissues and to the bases of the gland cells—the latter have been recorded by anatomists as possible secretory nerves (56), (56a). The gland is richly supplied with vasomotor nerves both constrictor and dilator (54), (55). The question of secretory nerves has attracted much attention during the last decade and still remains undecided. All the evidence is indirect. Asher and Flack (49), (50) first showed that in rabbits the blood pressure response to a given dose of adrenalin was greater after electrical stimulation of the superior laryngeal nerve with intact thyroid than before such stimulation. They also showed that after thyroid removal, stimulation of the superior laryngeal nerve did not modify the blood pressure response to a similar dose of adrenalin. Oswald (51) obtained similar results by injecting iodothyroglobulin instead of stimulating the thyroid nerves. Levy (52) has confirmed Asher and Flack's work on cats. Epinephrin produces all these effects and also markedly raises the heat production in the absence of the thyroid gland. The difference is only one of degree. Von Cyon (53) has criticized Asher and Flack's work by pointing out that where blood pressure is used as an indicator, many factors particularly the degree of anesthesia must be carefully controlled, and also one must distinguish between goiterous and non-goiterous rabbits for this kind of work. Rahe, Rogers, Fawcett, Beebe and others

(58), (59) have shown that the iodine content of the thyroid may be reduced by prolonged faradic stimulation of the cervical sympathetic. Cannon and Cattell (57) showed that there was an alteration in the electrical potential of the gland on stimulating the cervical sympathetic. No such effect was observed when the vagus or sciatic nerves were stimulated or when the systemic blood pressure was changed. They interpreted this as evidence of secretory innervation.

All these methods are highly indirect and complicated and the results are not susceptible of complete analysis at present. Other more direct proof must be offered before one can accept the doctrine of secretory nerve control of the thyroid secretion. That some sort of nervous control whether secretory or regulatory exists is probable on the grounds of analogy. Whether this regulation is dependent on specific nerves or on specific chemical changes acting through a more general regulatory nervous mechanism is still to be demonstrated. On the other hand, there is very direct evidence that specific nerve endings are not necessary for thyroid tissues to show many evidences of variation in secretory activity. Thus, by autografting the thyroid in widely separated parts of the body it has been shown that such transplanted thyroid wherever located shows chemical and morphological changes paralleling those of the non-transplanted tissue (60). If the transplant is undergoing hyperplasia, the non-transplanted portion is also undergoing changes similar in nature and degree. The administration of iodine inhibits hypertrophy alike in the transplanted and non-transplanted thyroid. In 1914 Cannon (61), utilizing Langley and Anderson's (62) method of anastomosing motor and sympathetic nerves, united the anterior root of the phrenic with the peripheral end of the cervical sympathetic in the cat and reported the occurrence of symptoms closely resembling exophthalmic goiter—tachycardia, emaciation, increase in metabolism to plus 100 per cent. He ascribed these phenomena to the bombardment of the thyroid with impulses discharged through the phrenic stump. These results have neither been confirmed by others (63), (64), (65) nor repeated by Cannon.

PHYSIOLOGY. *a. Effects of thyroid removal.* Our knowledge of the physiology of the thyroid may be said to have begun with Gull's report in 1874, *On a Cretinoid State Supervening in Adult Life in Women* (74). Prior to its publication, the function of the thyroid was either speculative—that it aided in the formation of erythrocytes, that it acted as the vascular shunt for the cerebral circulation, that it neutralized toxins, that it served only to give form to the neck, etc.,—or was hopelessly

confused with the function of the parathyroids. It was not until Gley in 1891 (75) rediscovered the parathyroids that any real differentiation of the functions of the thyroid and parathyroids was possible, and it was some twenty years after this that their functions were finally separated to the satisfaction of all workers.

Independent observations reported by the Brothers Reverdin in 1882 (76), and more clearly by T. Kocher in 1883 (77), on the effects of total thyroidectomy in man for goiter established the first experimental confirmation of Gull's clinical-pathological observations. Some of these operated human cases developed parathyroid tetany and died, while others developed during the next thirty to sixty days a cachexia which these surgeons recognized as similar to that described by Gull. The Reverdins designated the symptoms complex as operative myxedema (Ord (78) in 1878 having given the name myxedema to the condition described by Gull because his chemical examinations indicated there was an increased mucin formation in the thickened subcutaneous tissue) while Kocher called it *cachexia strumapriva*. Horsley (79), (80) in 1884, working with monkeys, observed a few instances of cachexia strumapriva; most of the monkeys developed tetany. Other observers carried out similar experiments on rabbits, sheep, goats, dogs, cats and guinea pigs. Those working with rabbits claimed that thyroidectomy was usually without noteworthy effect, while those working with carnivora usually obtained parathyroid tetany. The experimental thyroidectomies before 1891 in general added confusion rather than facts to the function of the thyroid. Since 1891 many species of mammals have been subjected to thyroidectomy using standard surgical technique and excluding the parathyroid factor. The most striking effect of thyroidectomy is a reduction in the total metabolism. In cats and rabbits this decrease begins usually in from six to eight days after thyroidectomy and reaches its lowest level between the twentieth and thirtieth day (81), (82). This low level of metabolism may be maintained for years (rabbit) or as accessories develop the metabolism may gradually rise to normal. In these animals the average maximum reduction in metabolism is around 35 per cent to 40 per cent, which corresponds closely to that observed in the severest forms of human cretinism and myxedema and may be designated as the myxedema level.

While qualitatively the symptoms following thyroidectomy in both young and adult animals are similar, the visible manifestations are strikingly more prominent in animals thyroidectomized during the period of growth. Adult herbivora like the rabbit, sheep or goat may

show very little change clinically beyond a dryness and thickening in the skin, thinning of the hair, a gain in weight and a lowering of body temperature. This was observed by the earlier workers. Heat production measurements in these animals, however, show the usual marked decrease. In the young there are in addition the gross manifestations of stunted physical, mental and sexual development.

With a knowledge of the frequency and functional importance of accessory and aberrant thyroid tissue, the writer believes that in the adult animal (sheep, rabbit, cat) the thyroid is not essential for vegetative life, while in the young it is only indirectly essential in that it is necessary for growth and development. The thyroid is an organ acquired late in the development of animal life, present only in the higher Chordates, and all we know of its function indicates that it provides the means for maintaining a higher level of metabolism and for varying its rate.

*b. Biochemistry.* The next most important advance in thyroid physiology was the demonstration in 1891 by Murray (82a), of the remarkable therapeutic effect of the injection of a glycerol extract of fresh sheep's thyroid in cases of Gull's disease. This was quickly followed by the independent announcements in 1892 by Howitz (83), by Mackenzie (84) and by Fox (85), that thyroid either fresh or dried or boiled was equally efficacious when fed by mouth. Emminghaus and Reinhold (86) in 1894 showed that thyroid feeding also caused a marked reduction in the size of goiter. These discoveries of the therapeutic effects of thyroid feeding also mark the beginning of thyroid biochemistry. The names of Hutchinson (87), (88), (89) in Great Britain, Frankel (90) in Austria, Baumann, Roos (91), Oswald (92) and Dréchsel (93) are most closely associated with the biochemical work of this early period which culminated in the announcement in 1895 by Baumann (94), (95), (96) of Freiburg that iodine in a rather firm organic combination was a normal constituent of the mammalian thyroid. He obtained a substance by acid hydrolysis which he named "iodothyrene." This substance was later put on the market by a pharmaceutical firm under the name of "thyroidin." The substance obtained by Baumann was a brown amorphous powder insoluble in water and acids, slightly soluble in alkalis, gave no protein reactions, contained 0.4 to 0.5 per cent P. and as high as 9.3 per cent iodine. Later work has shown that this method of hydrolysis (10 per cent  $H_2SO_4$ ) partially destroys the specific iodine compound. Baumann and his pupils, Roos and Goldman (97), showed that iodine was present in the

mammalian thyroid in very variable amounts and that feeding iodine increased the store. Oswald (98), (99), in 1899, observed that the iodine was contained in the colloid, and that the colloid of the thyroid was mainly globulin, and introduced the terms thyreoglobulin and iodothyreoglobulin. He showed that the iodine content varied in general with the amount of visible colloid in the glands, but that hyperplastic glands could be rich in globulin and iodine-free. The relation of iodine to the structure of the thyroid has been particularly studied by Marine and Williams (100) and Marine and Lenhart (101). These studies have cleared up the controversy that had developed concerning the fundamental importance of iodine in thyroid physiology, because many workers noting the absence of iodine in the thyroid in certain conditions still claimed it was only an accidental constituent probably excreted into the thyroid as a waste product. Much of the earlier work claiming the absence of iodine in the thyroid was, of course, due to faulty methods of chemical analysis. The final results in all of this work showed that the iodine store in the thyroid varies in general with the amount of stainable colloid, inversely with the degree of active hyperplasia and in the extreme degrees of active hyperplasia seen in cretinoid states in man and animals the iodine store may be entirely exhausted. In the following table the relation of iodine to histological structure as found by Marine and Lenhart are given:

	NORMAL	EARLY HYPERPLASTIC STAGE	MODERATE HYPERPLASTIC STAGE	MARKED HYPERPLASTIC STAGE	COLLOID OR RESTING STAGE
Man.....	2.17*	0.88	0.71	0.32	2.00
Dog.....	3.32	0.62	0.37	0.11	1.99
Sheep.....	2.47		0.40	0.01	3.00
Ox.....	3.46	1.65		0.19	
Pig.....	2.51	1.10			2.35

\* Iodine in milligrams per gram dried gland.

Earlier workers reported discordant results regarding the presence of iodine in the fetal thyroid (102). Fenger (103), (104), using standard methods of analysis, showed conclusively in a large series of animals that iodine is present in the fetal thyroid of cattle, pigs, sheep and man. In cattle it is present as early as the third month of intra-uterine life, that is, six months before birth. The iodine content gradually rises with the increasing age of the fetus and shows the same variations dependent upon structure as seen in extra-uterine life. The writer has

made similar observations on a large series of fetal thyroids of dogs. The iodine store in the thyroid shows striking seasonal variations, being highest in the early autumn (October) and lowest in the early spring (April) in this latitude (Seidell) (105), (106). The thyroid has an extraordinary affinity for iodine (107), (108). This was first observed by Baumann in 1895, though iodine has been knowingly used in the treatment of goiter since 1820 (Coindet) and unknowingly throughout many parts of the world, civilized and uncivilized, for unknown centuries. Iodine given to pregnant mothers is also readily stored in the fetal thyroid. The amount of iodine taken up by a given thyroid varies with the degree of active thyroid hyperplasia. The maximum store per gram being relatively constant, for most mammals thus far examined averaging between 5 and 5.5 mgm. per gram of dried thyroid (109). The minimum amount necessary for the maintenance of normal gland structure is likewise relatively constant, averaging about 1 mgm. per gram of dried substance (110). The average normal iodine content for human thyroid is around 2 mgm. per gram of dried substance and the maximum total store of iodine in a strictly normal human thyroid does not exceed 25 mgm. (111), (112), (113), (114). These facts are of the utmost importance in the practical application of iodine to the prevention of goiter.

Perfused surviving thyroids show the same marked ability to take out and store iodine from the circulating fluid as is seen in the thyroid *in situ* (115), (116), (122). It has been shown that the iodine content of the thyroid of a dog may be increased several hundred per cent within five minutes after the injection of 50 mgm. of potassium iodide into the femoral vein. As much as 18.5 per cent of a single intake of 38 mgm. (50 mgm. KI) given to a dog by mouth may be recovered from the thyroid whose ratio to body weight was as 1:687. The thyroid stands alone at present among the specific affinities of tissues for inorganic substances.

The older literature contains many reports of the presence of appreciable amounts of iodine in the parathyroids, thymus, pituitary and other organs. Excluding its presence in tissue due to the recent administration or to contact with iodine, active normal tissues other than the thyroid do not contain amounts greater than could be accounted for by its discharge from the thyroid. A great deal of work has been done in the attempt both to isolate and to synthesize the active iodine compound of the thyroid, especially by Oswald (117), Nürnberg (118), Koch (119) and many others (120), (121). Whole proteins—casein, glutelin, serum-

albumin and globulin and many amino acids including tyrosin, tryptophane, histidin and phenylanilin have been iodized but none of these were active. Later work, using the tadpole as a test object, showed that iodized blood serum, especially the globulin fraction, has a slight thyroid-like effect (123). In 1916 Kendall (124) reported the isolation of the specific iodine compound in crystalline form which he named "thyroxin." According to his latest report (125) this substance has the empirical formula  $C_{11}H_{10}O_3NI_3$  and structurally is trihydro-triiodo-oxy-beta indolepropionic acid. This substance in the purest form yet obtained contains 65 per cent iodine, has a melting point of around 250 and crystallizes in sheaves of needles. Kendall has shown that this substance produces the same pharmacological effects as whole thyroid. Kendall also showed that iodine is present in the thyroid in both an active and inactive form (126). Taking advantage of the extraordinary affinity of the thyroid for iodine and of the Gudernatsch tadpole test, Marine and Rogoff (127) carried out experiments to determine the rapidity of the production of active thyroid iodine. Differences in pharmacological activity of the thyroid were detectable in eight hours after intravenous injection of 50 mgm. KI and the differences in activity were quite marked after thirty hours. These observations indicate that while the storage of iodine is instantaneous the formation of thyroxin is a relatively slow process.

In 1895 Magnus-Levy (128), (129) reported in Gull's disease that the respiratory exchange was markedly decreased below normal and that in Graves' disease it was notably increased. He also demonstrated that feeding thyroid to cases of Gull's disease markedly increased their respiratory exchange and the excretion of urinary nitrogen. Friedrich Müller had recognized the increased nitrogen excretion in Graves' disease in 1893 (130). These discoveries by Magnus-Levy are the most important contributions to the pharmacology of thyroid substance and among the most important contributions to our knowledge of thyroid physiology. The most characteristic pharmacological action of thyroid or of its isolated active substance, thyroxin, is an increase in total metabolism (131), (132), (133), (134), (135), (136), (137). Its action is in general proportional to its iodine content as determined either by measurements of heat production, nitrogen excretion or the Gudernatsch tadpole test (138) (the most sensitive test yet developed for thyroid). In tadpoles thyroid substance causes a rapid loss in weight associated with metamorphosis in a few days. This is proportional to the active iodine (139). Some have considered that the action

of thyroid on tadpoles was a specific stimulus to differentiation. All the phenomena observed may be explained on the well-known action of the thyroid in accelerating metabolism and the apparent specificity does not depend on a new specific action of thyroid but on its application to a living organism at a specific period in its development (140). The acetonitrile test of Hunt and Seidell (141), (142), (143) is not specific for thyroid activity, since while thyroid feeding increases the resistance of white mice to acetonitrile poisoning it decreases it in rats and guinea pigs. Further, removal of the thyroid does not alter the response in mice and the blood of thyroidectomized animals also protects. In man, Plummer (144) has roughly estimated that for the normal individual approximately 1 mgm. of thyroxin daily is sufficient for normal metabolic activity.

The effect of thyroid on the heart and circulation has been particularly studied by von Fürth (145), (146), von Cyon (53) and Oswald (147). Aqueous extracts of the whole gland injected intravenously caused the usual lowering of blood pressure, while purified solution of iodothyreoglobulin causes only a slight lowering of blood pressure but the heart rate is notably increased after a latent period. Oswald believes that thyroid increases the irritability of all sympathetic nerve endings. The thyroid has no specific effect on blood coagulation. In Graves' disease the coagulation time is usually prolonged but attempts to establish a thyroid relation have been negative (148), (149), (150). The relation of the thyroid to immunity has received a great deal of attention and the literature is confusing and contradictory. In general it has been found that hemolysin and agglutinin formation are higher in thyroidectomized than in control rabbits, while antitoxin (diphtheria) formation is lower in thyroidectomized animals (dog, horse and rabbit) (151), (152), (153), (154), (158), (159), (160). Fjeldstadt (155) in eighteen thyroidectomized rabbits found no increase in agglutinin formation. Most observers have reported numerous exceptions to the above general statement except Ecker and Goldblatt (156) who found the hemolytic titer of thyroidectomized rabbits always higher than the controls. It is stated that anaphylactic shock does not occur in guinea pigs if sensitized after thyroidectomy but does occur if sensitized before thyroidectomy (157). At present the results obtained do not warrant any direct association of the thyroid with antibody formation. The reaction to infections as shown by a reduction in iodine store and a tendency to hypertrophy and hyperplasia clearly indicate the thyroid is an important indirect factor in resistance

to infections. The increased heat production in infections is to some extent dependent upon the thyroid.

*c. Regeneration and transplantation.* The mammalian and avian thyroid regenerates rapidly after partial removal. Two major factors—the amount removed and the administration or the withholding of iodine, and probably many minor factors—age, diet, species, determine the degree of regeneration (70). In the dog, if one removes three-fourths of the gland, ordinarily regeneration occurs in the remaining fourth, but if small amounts of iodine are given, such regeneration does not take place. If as much as nine-tenths of the gland is removed, iodine in any amount does not protect against regeneration. Halsted (68) made an extensive study of thyroid regeneration in 1889. Ribbert (69) showed that regeneration may begin within a few days after partial removal and occurs first in the sub-capsular zone. His suggestion that this centrifugal growth is dependent upon a more active blood supply is probably correct. The irregular insular hyperthrophy and hyperplasia often seen in human goiter may be thus explained. Anatomically and chemically the thyroid changes in regeneration are identical with those occurring in the spontaneous hyperplasias of simple goiter and are controllable by the same methods, i.e., cellular hypertrophy and hyperplasia do not occur until after the iodine store falls below a given level (0.1 mgm. per gm. dried) (70).

Transplantation of the thyroid has been extensively studied by Cristiani (71), L. Loeb (72) and his co-workers, and by Manley and Marine (73). Thyroid tissue autografts readily in any part of the body and shows all the chemical and morphological reactions seen in the non-transplanted tissue. Growth of the transplant varies inversely with the degree of thyroid insufficiency created in the host. The administration of iodine or desiccated thyroid inhibits the growth of thyroid transplants. In much of the older work on transplantation, attempts to transplant large pieces, even whole glands, were failures. As only the peripheral layer of not more than four to six cells in thickness survive, the ideal transplant is a slice of tissue about 50 microns in thickness laid on some flat surface, as the subcutaneous tissue or the sheaths of muscles. The frozen thyroid tissue of rabbits also transplants readily (Manley and Marine). Many such experiments were made where the tissue was frozen with carbon dioxide from one to five minutes. The temperatures reached were not measured. Homeografts are rarely permanent. Barring technical errors, they all “take” but begin to undergo absorption as early as the seventh or eighth day.

Some animals destroy initial homeografts much more slowly than this, indicating that there are different degrees of foreignness of the transplanted proteins in animals as well as in man. In man, by transplanting within the same blood group it is probable that the average life of homeografts might be somewhat prolonged. But there are such great differences within a given group which cannot be detected by the usual hemolysin or agglutinin tests, that permanent value from homeo-transplantation is at present hopeless and must continue to be until some means is discovered to overcome the foreign protein reaction to the grafted tissue.

Heterotransplantation of thyroid in mammals never succeeds.

*d. Diet.* Diet notably affects both the structure and chemistry (166), (167), (168), (169). Baumann (96) in 1896 and many others (161) noted in dogs that fresh meats caused hypertrophy of the thyroid, while sea fish (cod) increased the iodine store. Watson (162) also found that a meat diet caused hypertrophy and hyperplasia of the thyroid cells in rats. Marine and Lenhart (163) (164), (165) showed that liver, particularly pigs' liver, was the most potent of a great variety of meats in causing thyroid hyperplasia in dogs and cats and also this food was an important factor in the causation of goiter in brook trout. Recent work by McCarrison (170), confirmed by Mellanby (171) showed that fats were even more potent in producing thyroid hyperplasia. McCarrison's suggestion that his effect is in part dependent on an increased bacterial putrefaction in the intestine seems unlikely. As thyroid hyperplasia is secondary to the depletion of the iodine store, these facts indicate that diets rich in proteins and fat increase the rate of discharge of iodine. It seems probable that thyroid activity is more necessary for the oxidation of fats and of proteins than of carbohydrates. Carbohydrate diets do not cause thyroid hypertrophy, as has been shown by McCarrison. Inanition brings about involution of the thyroid, decrease in the size of the epithelial cells and increase in colloid (172), evidence of decreased functional activity.

**INTERRELATIONS.** We are only at the beginning of definite knowledge concerning its functional interrelations with other tissues. During the last decade this subject has become involved in a stupendous mass of ill-advised speculation, exploitation and fiction. Interrelations may be either inhibitory (antagonistic) or augmentory in nature. Sufficient facts are available to indicate that these correlations determine the thyroid's most important effects on nutrition. These effects are brought about by acceleration and inhibition of its functional activity

which in turn are caused by chemical factors, positive or negative, acting through the blood stream either directly on the gland cells or indirectly through nerve impulses. Further, we must recognize that inhibition and acceleration of tissue activity may be brought about by the presence of a specific hormone or by its absence and that apparent acceleration may be an actual loss of inhibition and vice versa.

*Thyroid-Parasex glands.* (*Suprarenal cortex, interstitial and luteal cells.*) The relation of the thyroid to the sex glands was known to the ancients in its crudest external manifestations—the thyroid enlargement with menstruation, puberty, pregnancy and menopause (173). This relationship has passed down to our time with no proved additions to our knowledge concerning it (174). Recently it has been demonstrated that when the suprarenal cortex in rabbits is sufficiently injured as by freezing or by partial removal, a marked chronic increase in heat production usually occurs (80 per cent in a series by Marine and Baumann) (175). This increase usually begins within three to six days after the suprarenal injury and may last from two weeks to several months. Heat production may be increased to 60 per cent or more above the normal. On the other hand if the thyroid gland is removed and the metabolism allowed to fall to the myxedema level prior to the injury to the suprarenal cortex, this increase in heat production does not occur (82). Scott (176) has confirmed these findings, using cats. Golyakowski (177) in 1899 in a brief preliminary report observed increased  $\text{CO}_2$  output in dogs following ligation of the suprarenal vessels. There is some evidence that the increased heat production is associated with a loss of iodine from the thyroid and recently Black, Hupper and Rogers (178) have published evidence that feeding "suprarenal gland residue" to dogs increased the iodine store of the thyroid. This reaction with increased heat production appears then to be a suprarenal cortex thyroid interrelationship. Our present interpretation is that the suprarenal cortex exercises a regulatory or inhibitory control over thyroid activity and when this is withdrawn the thyroid automatically responds with increased function. It should be pointed out that there is evidence that the suprarenal cortex exercises an inhibitory control over other tissue functions as well—the thyroid suprarenal interrelation, therefore, is not an isolated one. The practical application of these observations may be of great importance; for example the enlargement of the thyroid at puberty, during menstruation, pregnancy and menopause may be thus partly explained. The effect of bacterial toxins in causing thyroid hyperplasia may be in part determined by a primary

injury to the suprarenal cortex. Other well-known facts involving obvious interrelations as, for example, the hypersusceptibility of certain individuals to adrenalin or the hypersusceptibility of certain individuals to desiccated thyroid and thyroxin, probably have as their basis this fundamental thyroid suprarenal cortex interrelationship. Exophthalmic goiter is in some way intimately involved in this interrelation and the popular conception that this disease is a primary thyroid disease must give way to a broader conception in which cortical exhaustion indirectly, and epinephrin stimulation directly, are in my opinion important primary factors in bringing about increased thyroid activity. Enlargement of the suprarenal cortex with an increase in the epinephrin store has been observed following prolonged feeding with desiccated thyroid (179), (180), (181), (182), (183). During starvation, enlargement of the suprarenal cortex has been observed. Both of these phenomena may be interpreted as an attempt to suppress thyroid activity.

Finally, the normal involution of the suprarenal cortex in infants should be mentioned. This remarkable destruction of the reticular and fascicular zones of the cortex has been observed only in infants and begins during the second or third week of extra-uterine life (183a). The process is initiated as a hemorrhagic infiltration of the two inner zones and goes on to necrosis, destruction and absorption of these layers with collapse and folding of the glomerular zone on to the medulla. The duration of the stages of absorption and healing is indefinite. Some authors estimate it at two to three weeks and other at two to three months. The end result, however, of this destruction is a marked decrease in the volume of cortex so that a child one year old has a smaller total volume of cortex than at birth.

The physiological significance of this rapidly progressive partial destruction of the cortex is unknown. It is not accidental or traumatic. Its occurrence in accessory suprarenals as well suggests that it is a systemic purposeful reaction to meet the altered conditions incident to extra-uterine life. In the light of the relation of experimental injury of the suprarenal cortex in rabbits, dogs and cats to increased heat production, it is suggested that one of the effects of the cortical destruction in infants may be increased heat production through thyroid activation. All that can be said at present is that the cortical injury and increased heat production in infants begin at approximately the same time and parallel each other.

*Thyroid-Chromophil tissue.* Epinephrin injected intravenously causes a marked constriction of the thyroid vessels. An interrelationship of

function was first postulated by Eppinger, Falta and Rudinger (184). They assumed that the chromophil system directly stimulated the thyroid. This conception received experimental support from the work of Asher and his pupils (186), who in 1910 showed that the blood pressure response in rabbits to a given dose of adrenalin was greater after stimulation of the thyroid nerves with intact thyroid than before such stimulation. This has been confirmed from several sources and especially by Cannon and his co-workers. The Goetsch epinephrin test in exophthalmic goiter is a clinical application of this reaction. Oswald has shown that a similar increase in the reaction to epinephrin may be obtained by injecting iodothyreoglobulin instead of stimulation of the thyroid nerves. The nature of this reaction is still in doubt (187), (188). Asher and Flack believe that the thyroid hormone increases the irritability of or sensitizes the tissues innervated by the sympathetic nervous system in some way so that it is more susceptible to stimulation by epinephrin.

*Thyroid-Gonad interrelationship.* Very little is known (185). That there is an important direct relation between some constituent of the gonads, especially in the female, and the thyroid is certain. The exceedingly complex nature of the sex gland has thus far been a perfect barrier to trustworthy experimental investigation. Total removal of the gonads usually leads to a slight depression of the thyroid function (189).

*Thyroid-Hypophysis.* Rogowitsch (190) and others (191) (192), (195), (196) have reported marked enlargements of the anterior lobe and especially the pars intermedia following thyroidectomy—as much as 400 per cent. They interpreted this enlargement as indicating that the pituitary could function vicariously for the thyroid. Subsequent work by Simpson and Hunter (193) and by the author has not confirmed this. In rabbits there is a slight hypertrophy of the anterior lobe, but this is rarely more than 15 to 20 per cent after five or six months. Many investigators have found traces of iodine in both the human and sheep hypophysis—others have failed to find it. Simpson and Hunter (194) showed conclusively that the sheep pituitary contained no iodine even in animals recently fed with this element. Livingston (197) on the other hand has published a few observations indicating that desiccated thyroid protects male thyroidectomized rabbits against pituitary hypertrophy. No pituitary hypertrophy was observed in thyroidectomized female rabbits even without thyroid feeding. Hewitt (198), however, reported that thyroid feeding in white rats caused pituitary

enlargement. The idea that the thyroid and pituitary were functionally related seems to have been suggested by Virchow and was based on the morphological resemblance of the colloid filled follicles of the para intermedia to the thyroid follicle. Acromegaly is usually associated with a slight increase in the size of the thyroid and with an increase in heat production rarely over 20 per cent. None of the facts thus far established suggest any direct functional relation between the hypophysis and the thyroid.

*Thyroid-Thymus.* There is no evidence of any important relation between these two organs despite a relatively large literature dealing with their functional interdependencies (199). There is no doubt that the thymus is usually enlarged or persistent in many conditions in which the thyroid is involved, for example, simple goiter, myxedema, Graves' disease. Asher and Ruchti (200) found no change in the respiratory exchange after thymectomy in rabbits whether performed before or after thyroidectomy. Gudernatsch thought thymus feeding inhibited to some extent the action of the thyroid feeding on tadpoles. Baumann (unpublished) has found that foods enriched by the addition of protein-free nucleic acids of any origin stimulate growth in tadpoles. That there is an important indirect relation between the thyroid and thymus through the sex glands and suprarenals is certain since each of these glands is closely associated functionally with the sex and parasex tissues, and both the thyroid and the thymus are usually affected in conditions involving the suprarenals or sex glands, as for example, Addison's disease, status lymphaticus, castration, Graves' disease, etc.

*Thyroid-Spleen.* Asher and his pupils (201) have recently revived the question of the thyroid-spleen interrelationship suggested by Tauber (202) in 1884. Asher found that splenectomized rats with intact thyroids were less resistant to reduced oxygen pressures than normal rats, or rats with combined thyroidectomy and splenectomy. Splenectomy has been found to slightly increase the respiratory exchange in rabbits but this also occurs when the thyroid is removed.

*Thyroid-Pancreas and Liver.* Falta thought the thyroid and pancreas were antagonistic (203). He stated that an epinephrin injection which in normal dogs caused a marked glycosuria does not produce glycosuria in thyroidectomized dogs. Similar observations were made by Grey and de Sautelle (204) on dogs and by Pick and Pineles on goats. Underhill (205), however, denies that thyroidectomized dogs in which great care has been exercised to preserve the two external parathyroids are less susceptible to adrenalin glycosuria. Thyroid feeding was found

to produce a marked decrease in the diastatic activity of the pancreas of white rats and this was often associated with enlargement of the pancreas (206). Clinicians have reported the frequent association of some of the symptoms of Graves' disease with acute pancreatitis (207). It has also been suggested that the lowered sugar tolerance and glycosuria of Graves' disease might involve a thyroid-pancreas interrelation. The increased alimentary tolerance for glucose in myxedema or after thyroidectomy is of doubtful significance. It may be due to decreased absorption from the intestine rather than to a direct thyroidectomy influence. What evidence there is seems to indicate that any thyroid-pancreas interrelationship is an indirect one and dependent on epinephrin sensitization. Whipple and Christman (208) have shown that thyroidectomy does not influence the excretion of phenoltetrachlorophthalein by the liver.

*Thyroid-Parathyroids.* No interrelation of function is known. The earlier affirmative statements were based on faulty methods and errors in interpretation.

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## INTRACELLULAR DIGESTION

### THE ENZYMES AND ANTI-ENZYMES CONCERNED

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All of the protozoa and many somewhat higher animals obtain their food by intracellular digestion. Among the lower metazoa including sponges, coelenterates and some of the lowest forms of worms, such as turbellaria, cells lining the digestive cavity act as phagocytes, ingest solid particles of food and dissolve them usually within vacuoles in their cytoplasm. Among the coelenterates and turbellaria food undergoes preparatory changes within the enteric cavity but the part of enzymes poured out into the cavity is doubtful. Extracellular digestion by enzymes secreted into a digestive tract is first definitely established in the ascending animal scale in the higher worms, such as nematodes, earth worms, etc., and becomes the sole method for the digestion of food.<sup>1</sup>

A proteolytic enzyme has been extracted from the bodies of amoebae and from the phagocytic cells of coelenterates, such as the sea anemones; it digests protein in a weakly acid and in a weakly alkaline medium. When the amoeba or the digesting phagocytes of higher forms ingest particles of blue litmus they are quickly turned to red but after a time the vacuole surrounding the granule loses its free acid and the indicator shows a neutral or alkaline reaction and many other indicators exhibit similar reactions.

In the lowest metazoa, namely, in the sponges, and in all higher forms which he examined, Metschnikoff (67) found mesoblastic phagocytes capable of approaching and ingesting foreign material which has found its way into the tissues of the organism. Among mammals the cells which exhibit these properties are notably the polynuclear leucocytes of the bone marrow and blood and certain mononuclear wandering cells which are widely scattered in the tissues. These cells approach

<sup>1</sup> The literature of phagocytic digestion of food in lower animals has been reviewed by O. von Fürth, *Vergleichende chemische Physiologie der niederen Tiere*, Jena, 1903 and by H. Jordan, *Vergleichende Physiologie wirbelloser Tiere*, Jena, 1913.

or fix foreign particulate matter which has entered the body, engulf it and so far as they are capable bring about the solution of the ingested material. Not only is foreign material subject to the activities of these cells but body substance, including cells and intercellular substance, which has been injured or killed, may undergo some change as the result of which it becomes the prey of the phagocytic cells and is eliminated by intracellular digestion.

When phagocytic cells of the higher animals are allowed to ingest indicators, such as litmus, tournesol-blue or alazarin sulphate, no change occurs. With neutral-red, bacteria and the nuclei of cellular elements ingested both by polynuclear leucocytes and by macrophages assume a brownish red color which Metschnikoff has attributed to a feebly acid reaction.

**ENZYME OF THE POLYNUCLEAR LEUCOCYTES; LEUCOPROTEASE.** The presence of peptone in pus was observed by Eichwald (16) in 1864, and later this observation was confirmed by Maixner (66) and by Hofmeister (31). In association with diseases characterized by pus formation, namely, empyema, purulent peritonitis, cerebro-spinal meningitis, etc., Maixner found peptone in the urine. The occurrence of proteolytic enzymes in the cells of purulent exudates was first demonstrated by Friedrich Müller (76), who showed that a glycerine extract of purulent sputum digests fibrin or coagulated protein in the presence of a weakly alkaline reaction. Similar enzyme was demonstrable in fresh pus from an acute abscess but was absent in the thin fluid from a "cold abscess." Leber (60) and later Achalmé (1) showed that pus liquefied gelatin, and dissolved fibrin, egg albumin coagulated by heat, and casein.

*Methods.* Many methods used for the study of pepsin and trypsin are not applicable to the weaker enzymes present in phagocytic cells. Fresh fibrin frequently employed is not well adapted to the study because it contains enzymes which bring about autolysis. Some very simple methods have been introduced with the hope that they would be useful to physicians and numerous clinical studies have been made with their aid. The methods which have been most used will be cited briefly.

**Liquefaction of gelatin:** This method introduced by Fermi (21) has been used by Eppenstein (18) for the demonstration of enzymes in leucemic blood. Gelatin in the strength of 6 to 8 per cent is used as a substrate for the demonstration of enzymes. The substance to be examined is mixed in quantity of 0.2 to 0.5 cc. with 1 to 2 cc. of gelatin

containing 1 per cent soda and kept in an incubator at 37° to 40°C. during 12 or more hours. Should digestion occur the gelatin remains liquid after cooling with ice during several hours.

Digestion of casein: Gross (25) and Fuld (23) have introduced a method for the study of tryptic digestion which has been much employed in the study of the anti-enzymotic activity of the blood serum. Casein in 1 per cent solution in  $\frac{1}{10}$  N sodium hydroxide is neutralized with  $\frac{1}{10}$  N hydrochloric acid and diluted with five times its volume of physiological salt solution. After the enzyme has acted upon this substrate the mixture is acidified by 5 per cent acetic acid in weak alcohol and if digestion is complete the solution remains clear, since the digestion products of casein are not precipitated in acid solution, but if undigested casein remains it is precipitated. Numerous studies of the anti-enzymotic activity of the blood serum with carcinoma and other disease have been made by this method. The amount of serum necessary to inhibit a given quantity of enzyme is determined.

Serum plate method: Eduard Müller and Joehmann (71) have used, for demonstration of proteolytic enzymes, Loeffler's medium spread out as solid plates in Petri dishes. The medium consists of two parts of beef serum and one part of bouillon containing approximately 1 per cent of glucose and coagulated by exposure to a temperature from 85 to 95°C. during several hours. A drop of the material under investigation is placed upon the surface of the plate which is then incubated at from 55° to 60°C. during 24 hours. At this temperature a shallow cup-like depression is formed by the action of the enzyme upon the coagulated beef serum. Excavation of the surface of the plate does not occur at body temperature and the higher temperature has the advantage that it prevents the multiplication of bacteria. Various dilutions of the fluid to be tested for enzymotic activity may be prepared and the titer at which digestion ceases may be determined. Many drops may be applied to the same plate. This somewhat crude method has been widely employed for the study of enzymes and anti-enzymes both in health and disease but it has in great part served to confirm observations made by more accurate quantitative methods. The observations made upon leucocytic enzymes and anti-enzymes by this method have been fully reviewed by Weins (102) but he has overlooked almost all of the observations made by quantitative chemical methods.

Measurement of digestion by nitrogen determination: When the enzyme digests a protein substrate the activity of digestion is meas-

ured by the amount of protein coagulable by heat or by chemical means which has been split into incoagulable products such as albumose, peptone and lower nitrogen-containing decomposition products. This measurement is readily made by determining the amount of nitrogen in incoagulable substances before and after digestion during 24 hours at 37°C., some conveniently obtainable protein such as blood serum denaturalized by heat being used as a substrate. Nitrogen may be determined by the Kjeldahl method which was used by Ascoli and Maresche (4) and by Opie (79), or by the microchemical method of Folin, which Jobling (49) used. The method ensures an accurate determination of the activity of digestion.

*Preparation and character of leucoprotease.* The enzyme of the polynuclear leucocytes was extracted (Friedrich Müller) from purulent sputum or fresh pus by means of glycerine. A permanent preparation of the enzyme may be obtained from the leucocytes of a sterile inflammatory exudate produced by the injection of aleuronat into the pleural cavity of a dog by treating the washed cells with absolute alcohol in sufficient quantity to cause dehydration (Opie). After removal of the alcohol the cells are treated with ether, dried and reduced to a fine powder. This material actively digests protein such as blood serum denaturalized after dilution with an equal volume of physiological salt solution by heating to 75°C. It acts in a neutral or alkaline (0.2 per cent sodium carbonate) solution but is almost wholly inactive in the presence of acid (0.2 per cent acetic acid). This enzyme is slightly increased in activity by short exposure to temperatures between 50 and 60°C., is slightly impaired by a temperature of 65°C. and is destroyed between 70 and 75°C.

Fresh leucocytes incubated on Loeffler's serum plates at 37°C. cause no cupping of the surface but if leucocytes are first subjected to an elevated temperature (55°C.) and then incubated at body temperature active solution of the plate occurs. The high temperature according to Müller and Jochmann is required to destroy the leucocytes so that their enzymes may be set free. The enzyme causes active proteolysis at temperature between 50° and 55°C.

The proteolytic enzyme of the polynuclear leucocytes has been given the name "leucoprotease" by Opie (81).

A purified enzyme was prepared from leucocytes by Jochmann and Lockemann (57). Material containing the enzyme was allowed to autolyze during from 24 to 48 hours at 55°C. The autolysate was then treated with about five times its volume of alcohol (2 parts) and ether

(1 part) in order to remove fatty material and to precipitate protein. After standing during one day, the material was filtered and the residue, first evaporated to get rid of alcohol and ether, was intimately rubbed with glycerine in the proportion of about one-fourth of the volume of the original material from which the enzyme was obtained, and an equal volume of water was added. After standing one or two days in the dark the solution was passed through a porcelain filter and the clear filtrate was treated with five or six times its volume of a mixture of alcohol and ether. The white precipitate thus formed was dried in a vacuum over concentrated sulphuric acid. The final product which was yellowish brown and somewhat hygroscopic formed a brown solution when dissolved by rubbing with water or physiological salt solution. It digests coagulated blood serum, fibrin, gelatin and casein best in a weakly alkaline solution. When dissolved in water it is destroyed by temperatures between  $70^{\circ}$  and  $75^{\circ}\text{C}$ . but in dry form though impaired in activity resists temperatures from  $75^{\circ}$  to  $95^{\circ}\text{C}$ . being destroyed by  $100^{\circ}\text{C}$ .

The effects of various chemicals upon the enzyme of leucocytes have been studied by several observers, including Jochmann and Lockemann (57), Müller and Kolaczek (75). The enzyme is especially resistant to the action of formalin. Jochmann and Ziegler (58) noted that the proteolytic enzyme of leucemic organs was not destroyed by preservation in 10 per cent formalin even after the lapse of a year. If part of a spleen is placed in salt solution and part in 10 per cent formalin (Jochmann and Lockemann) disintegration proceeds rapidly in the former at a temperature of  $55^{\circ}\text{C}$ . so that complete liquefaction has occurred after 48 hours, but in the presence of formalin the tissue is completely preserved. If tissue preserved in formalin is washed in running water and then applied to the serum plate, excavation of the surface occurs. The experiment shows that digestion is inhibited by the presence of formalin but the enzyme is not destroyed. The purified enzyme of Jochmann and Lockemann was impaired but not destroyed when dissolved in 10 per cent formalin. It is noteworthy that the action of formalin on trypsin is similar to that upon the enzyme of leucocytes.

The production of fever by leucoprotease was observed by Jochmann (54). He found elevation of temperature in rabbits following the injection of enzyme of leucocytes or of pancreatin into the vein or into the subcutaneous tissue; it appeared after one hour, the temperature remained somewhat elevated after two hours and then fell. The result

was the same when the solution was heated during a quarter of an hour at 80° to 90°C. and is not dependent upon the presence of the proteolytic enzyme but is caused by substances related to proteins and associated with the enzyme. Jochmann thinks that these substances, as well as the toxins of bacteria, are concerned in the production of the fever which accompanies suppurative processes.

Diminution of the coagulability of drawn blood by addition of enzyme of leucocytes has been observed by Jochmann (54). Very large quantities of the enzyme injected into an animal cause an initial diminution of coagulability and later an acceleration of coagulation.

*Occurrence of leucoprotease in the tissues.* The proteolytic enzyme of polynuclear leucocytes which like trypsin digests in the presence of an alkaline reaction has been found in those organs within which polynuclear leucocytes are particularly numerous. Leucoprotease was found in the bone marrow by Opie (80). Autolysis of liver, kidney, spleen, lymph nodes and other tissues proceeds more rapidly in the presence of weak acid (e.g., 0.2 per cent acetic acid) than in a neutral or alkaline medium. Bone marrow, on the contrary, autolyzes more actively in an alkaline (e.g., 0.2 per cent sodium carbonate) than in an acid medium and furthermore digests extraneous protein under the same conditions. E. Müller and Jochmann (71) found that a drop of the material which was pressed from the cut surface of a human lymph node failed to digest the surface of coagulated serum incubated at 50°C. but under the same conditions active digestion was caused by red marrow squeezed out of a rib or vertebra.

Leucoprotease is formed within the polynuclear leucocytes before they leave the bone marrow. Here they elaborate a proteolytic enzyme which in some respects resembles trypsin. Nothing is known concerning the formation of the enzyme with the leucocyte. A zymogen has not been found. No relation to the specific granules of the polynuclear leucocytes has been demonstrated.

Hedin and Rowland (28) found that the expressed juice of the spleen of beef, horse, pig and sheep undergoes much more active autolysis in an acid than in an alkaline medium. Nevertheless in the presence of an alkaline reaction (0.2 to 0.37 per cent sodium bicarbonate) digestion is still considerable. Hedin (26) succeeded in separating almost completely two enzymes one of which, designated lieno-B-protease, digests in an alkaline medium. These enzymes not only digest the cells of the spleen but cause the disintegration of other proteins such as fibrin, casein and coagulated blood serum.

By quantitative determinations by means of the Kjeldahl method, Opie (80) found that the digestive activity of spleen in the presence of an alkaline medium stood next to that of bone marrow and exceeded that of liver, kidney and lymphatic nodes.

The proteolytic enzyme of leucocytes has been found by Friederich Müller and subsequent investigators in purulent exudates from patients. The studies of Opie were in great part made with the active leucoprotease obtained from sterile inflammatory exudates of dogs. E. Müller and Jochmann (72) using the serum plate method, have found no solution of the serum with cells obtained from rabbits and guinea pigs by injecting a solution of nucleinic acid below the skin or into the peritoneal cavity. Using the same method, Jochmann and K. Ziegler (58) have observed proteolysis caused by spleen and bone marrow of monkeys and slight proteolysis caused by the same tissues from the dog but have found none when they tested organs from fox, cat, various rodents, pig, sheep, beef and horse. The serum plate method is not sufficiently delicate to determine the absence of enzyme. A weak proteolytic enzyme digesting in the presence of an alkaline medium was found by Opie and Barker (84) in leucocytes obtained by injecting turpentine into the subcutaneous tissue of a rabbit; digestion was measured by Kjeldahl determination of nitrogen in the products of digestion. Using Van Slyke's micro-method to measure amino nitrogen, Parker and Franke (87) found only a very small increase after digestion of purified blood albumin and came to the conclusion that leucocytes of rabbits contain no proteolytic enzyme; they found, however, erepsin, which formed amino acids by digestion of peptone. No enzyme resembling the leucoprotease of mammals was found by Opie and Barker (84) in the leucocytes, bone marrow or spleen of the hen.

*Relation of leucoprotease to trypsin.* Leucoprotease which acts in the presence of an alkaline medium resembles trypsin but extracts obtained from leucocytes are much less active than preparations of trypsin. Those who have assumed that the anti-enzyme of the serum is a true antibody have reached the conclusion that leucoprotease and trypsin are identical for as Jochmann and Kantorowicz (56) have found, injection of animals with one of the two substances increases the inhibiting action of the serum for both. Furthermore, serum of which the inhibiting action is overcome by addition of one enzyme no longer inhibits the other. This evidence is inconclusive if it can be shown that the anti-enzymotic activity of the serum is caused by non-specific substances. This subject will be discussed under "Antileuco-

protease" and it will be evident that the more recent investigators find that the anti-enzyme of the serum is not a specific antibody.

Wiens and E. Müller (103) found that the blood serum of the turtle failed to inhibit the human leucocytic enzyme but inhibited trypsin as effectively as human serum. Jochmann and Kantorowicz (55) found that turtle serum inhibited very slightly the purified leucocytic enzyme whereas it actively inhibited trypsin in from 5 to 10 per cent solution, but human serum also inhibited trypsin more completely than leucocytic enzyme.

By the action of purified enzyme from leucocytes upon Witte's peptone in the presence of sodium carbonate (less than 1.5 per cent) at 37°C. under chloroform, Jochmann and Lockemann (57) obtained crystals of trypsin after 5 or 6 days; parallel tests with pancreatin showed the presence of characteristic crystals after one day. Leucin, tryptophan and ammonia were demonstrable according to these observers as products of leucocytic digestion of peptone and they reach the conclusion that there is a very close similarity between the enzyme of leucocytes and trypsin.

A comparison has been made by Jobling and Strouse (49) between the products of digestion obtained from casein, purified egg albumin and Witte's peptone by the action of a solution prepared from dried powdered leucocytes on the one hand and of a solution made from the powdered trypsin of commerce on the other. The quantity of proteoses with leucoprotease exceeds that with trypsin whereas products of digestion below proteoses, namely, peptone and amino acids, were much less with leucoprotease than with trypsin. No tryptophan was formed by the action of leucoprotease. The digestion with leucoprotease in their experiments did not progress as far as tryptic digestion. They found in fresh pus cells an erepsin-like enzyme which was capable of splitting petpone.

*Leucoprotease and immunity.* Metchnikoff (68) proposed the name microcytase for complement or alexin which is concerned in bacteriolysis because he believed that it was derived from the polynuclear leucocytes and had the characters of a proteolytic enzyme. For complement which combines with antibody or amboceptor to cause hemolysis he suggested the name macrocytase believing that it was derived from macrophages. The effect of heat upon complement indicates that it is not identical with leucoprotease on the one hand nor with protease of macrophages on the other, since these substances resist temperatures which destroy complement.

Proteolytic enzyme of leucocytes was found to have no bactericidal action by Jochmann (53) when tested with *B. typhosus*, staphylococci and streptococci, nevertheless, according to Jochmann, it digested typhoid and colon bacilli just as quickly as it digested fibrin. Living bacteria resisted digestion and even multiplied actively in the solution of enzyme. The enzyme of leucocytes failed to cause hemolysis of red blood corpuscles. Jochmann found that the enzyme of leucocytes, unlike trypsin, failed to destroy diphtheria toxin.

The effect of leucoprotease upon pneumococci has been studied by Jobling and Strouse (50) and for control compared with bacterial autolysis in salt solution. After incubation during twenty-four hours the turbidity of the fluid containing leucoprotease is much diminished and within forty-eight hours the fluid is almost clear though the autolyzing suspension remains cloudy. In the presence of the enzyme pneumococci are reduced to shadows after twenty-four hours and are Gram-negative whereas the autolyzing microorganisms are normal in appearance and still Gram-positive. Simple chemical reactions such as total acidity and formol titration indicate that bacteria are split into cleavage products which are lower than those obtained by autolysis. Formol titration as an index of the formation of amino-acids furnished evidence that leucoprotease causes proteolysis of pneumococci.

It is noteworthy that Jobling and Peterson (39), employing Folin's microchemical method for the determination of nitrogen have found no evidence of proteolysis when immune serum and complement cause bacteriolysis of typhoid and colon bacilli although proteolysis was demonstrable when trypsin acted upon the same microorganisms; they have not described parallel experiments with leucoprotease. The clearing of a bacterial suspension under the influence of immune serum does not prove that proteolysis has occurred, for Jobling and Strouse (52) found no parallel between the clearing of a suspension and the accumulation of products of protein disintegration.

Some of the effects of leucoprotease upon typhoid bacilli have been studied by Jobling and Bull (32). Typhoid bacilli like *B. coli* injected into the circulating blood of dogs causes elevation of temperature, bloody diarrhea, coma and death, and the abdominal organs, particularly the intestine, are intensely congested. This toxic substance is not destroyed by heat but is precipitated with the coagulable proteins of the bacteria so that the filtrate after heating to 100°C. is not toxic. If an emulsion of bacilli is subjected to the action of leucoprotease during from 2 to 5 days, the toxic element is no longer precipitated by

coagulation but is found in the filtrate obtained after heating to 100°C. The toxic substance is not specific and similar observations were made with *B. coli*, meningococcus and *Staphylococcus aureus*.

Observations of Kantorowicz (59) indicate that the resistance of living bacteria to proteolytic enzymes is referable, not to vital characters, but to anti-enzyme which is present in the bodies of the bacteria. Gram-negative bacteria lose this inhibiting property when heated to 70°C. but Gram-positive bacteria inhibit digestion even after boiling. Dried organisms retain their anti-enzymotic activity but lose their resistance when extracted with acetone. Bacteria were used in the foregoing experiments of Kantorowicz as substrate for proteolytic enzymes and digestion was measured by clearing of the suspension. Jobling and Peterson (39) measured digestion by Folin's method of nitrogen determination. They found that dried organisms resisted digestion in a degree proportional to their content of unsaturated lipoids (see anti-enzyme for leucoprotease). Substances which extract lipoids reduce the resistance of bacteria to proteolysis and the saponified extracted lipoids inhibit the digestion of casein by trypsin in a degree proportional to their unsaturation.

Bacteria, namely typhoid and colon bacilli, were found by Jobling and Peterson (39) to be so altered by immune serum and complement that they are more readily digested by trypsin. Complement alone or an excess of immune serum and complement rendered bacteria more rather than less resistant to proteolysis. Rosenow (91) noted that the intracellular digestion of pneumococci ingested by leucocytes *in vitro* varied considerably when the blood of different individuals was employed and came to the conclusion that the variation was referable to the action of the serum on the leucocytes stimulating them to increased activity. Douglas (15) treated *B. pestis* which undergoes phagocytosis in the absence of serum with unheated and with heated serum and subjected them to phagocytosis. Bacilli which had been treated with fresh serum underwent active intracellular digestion whereas those treated with heated serum remained intact within the substance of the leucocyte. Douglas further found that bacteria (bacillus of Friedländer) or red blood corpuscles exposed to the action of fresh serum and then washed by centrifugalization were digested by trypsin or by leucoprotease whereas bacteria or red corpuscles treated with serum heated to 60°C. remained unchanged.

## INHIBITION OF LEUCOPROTEASE BY BLOOD SERUM; ANTILEUCOPROTEASE.

To describe the enzymotic activities of phagocytic cells it is necessary to discuss the inhibitory factors which are concerned. The anti-enzyme of the blood serum will be discussed in relation to leucoprotease and observations concerning inhibition of trypsin will be cited in so far as they help to explain the inhibition of leucocytic enzymes. The terms anti-enzyme, antileucoprotease, etc., are used as convenient descriptive words and do not imply that the substances which inhibit are specific antibodies. The antitryptic action of the blood serum was first observed by Hildebrandt (30).

The power of the blood serum to inhibit the proteolytic activity of leucoprotease was described by Opie (79). The anti-enzyme for leucoprotease passes from the blood into an inflammatory exudate and has an important part in the phenomena of inflammation (see p. 572). The addition of blood serum or serum of an inflammatory exudate to a mixture of leucoprotease and substrate, such as denaturalized serum, gelatin or fibrin, prevents or, in smaller quantity, retards proteolysis. A temperature of 75°C. maintained during one-half hour sufficed to destroy the anti-enzymotic activity of serum, which was not impaired by a temperature of 65°C. The anti-enzyme was more effective in an alkaline (0.2 per cent sodium carbonate) than in a neutral medium but its inhibitory action was lost in the presence of acid (0.2 per cent acetic acid).

Employing the serum plate method E. Müller and Jochmann (71) found that serum inhibited the enzyme of leucocytes. E. Müller and Kolaczek (75) found no anti-enzyme in the cerebrospinal fluid; none was found in the bile nor in milk. In normal urine they found no anti-enzyme but with abundant excretion of albumin especially with chronic passive congestion of the kidneys and with chronic parenchymatous nephritis the urine inhibited the enzyme of leucocytes.

The anti-enzyme of the blood is not specific for leucoprotease obtained from the same species. Opie and Barker (84) found that leucoprotease of the dog is inhibited by serum of man, ox, dog, cat, goat and rabbit, the last mentioned being stronger than the others. Leucoprotease of rabbit was inhibited by the serum of dog and of rabbit, that of the rabbit being again the stronger. The serum of birds (pigeon, hen) on the contrary failed to exhibit anti-enzymotic action when tested with the leucoprotease of dogs. Wiens and E. Müller (103) found that the serum of the turtle failed to inhibit the proteolytic enzyme of leucocytes but inhibits trypsin almost as effectively as human serum.

Antileucoprotease was precipitated by Opie and Barker (84) with the albumin fraction of the blood serum, obtained by complete saturation with ammonium sulphate but was absent in the euglobulin fraction obtained by one-third saturation or in the globulin fraction obtained by half saturation.

By adding increasing quantities of leucoprotease to the same quantity of serum Opie (82) found that a given quantity of serum can inhibit the action of only a limited quantity of enzyme; for example, 2.5 cc. of serum completely inhibited the proteolytic activity of 20 mgm. of dried leucocytes but failed to prevent proteolysis when a larger quantity of enzyme was employed.

*Anti-enzyme in the blood serum with disease.* Numerous studies of the anti-enzymotic activity of the blood have been made; from the standpoint of diagnosis they have disappointed early expectations. No exhaustive review of the extensive literature of this subject will be made. Observations of interest in relation to the enzymes of leucocytes will be cited briefly.

The anti-enzymotic activity of the blood was studied by Wiens (99), (100), (101) in a considerable number of diseases; he has used the serum plate method and has performed his tests with fresh pus. With general and local pyogenic infections he had found a diminution of anti-enzyme which he attributes to an increased destruction of leucocytes in the body. Wiens and Schlecht (104) have compared the anti-enzymotic strength of the serum with the number of leucocytes in the blood and with acute infectious diseases have usually found diminution of anti-enzyme during and immediately after an increase in the number of polynuclear leucocytes; later an increase of anti-enzyme may occur. Nevertheless variations in the anti-enzyme particularly in chronic disease may bear no relation to leucocytosis.

The anti-enzyme of the blood is not infrequently increased with conditions which are unaccompanied by leucocytosis. Brieger and Trebing (10), (11) found antitrypsin increased with carcinoma and sarcoma in 90 per cent of the cases examined, and later, observing the same change with some other diseases, for example, tuberculosis accompanied by wasting and cachexia designated this increase "the cachexia reaction." Brenner (9) found that the anti-enzymotic activity of the serum was usually increased with severe anemia and chlorosis but no definite relation to the number of red blood corpuscles nor to the hemoglobin content of the blood was evident. Increase of antitrypsin was found by K. Myer (69) in association with exophthalmic goiter. Nevertheless,

increase of antitrypsin is often unassociated with wasting and anemia. Gräfenberg (24) and later Thaler (98) and others found increase of anti-enzyme during pregnancy.

Sir A. E. Wright (106) has found coincident loss of antitrypsin and diminution of alkalinity in the edematous fluid at the site of infection with the gas bacillus of Welch both in man and in animals. In the blood diminution of alkalinity and increase of antitrypsin was found.

Following the administration of potassium iodide, Jobling and Petersen (41) found diminution of the antitryptic content of the blood serum, but in two instances of iodide poisoning antitrypsin was increased.

*The nature of the anti-enzyme in blood serum.* Increase of anti-enzymotic activity of the blood was found by Joemann and Kantorowicz (56) when they repeatedly injected subcutaneously into rabbits an extract of dried leucocytes from human pus. Before injection one drop of serum inhibited the proteolysis of one drop of pus but after these injections serum diluted from thirty-two to sixty-four times had the same effect. There was a parallel increase of anti-enzyme both for trypsin and for leucocytic enzyme. If trypsin was mixed with a quantity of serum which just served to inhibit its activity the mixture exerted no inhibitory action upon the enzyme of leucocytes. Upon the evidence of these experiments the authors reach the conclusion that the anti-enzyme for trypsin and that for the enzyme of leucocytes are identical.

Achalme (2) injected trypsin into the peritoneal cavity of guinea pigs and found the normal inhibitory action of the serum increased. After two months Bergmann and Bamberg (7) found the anti-enzymotic activity of the serum of dogs doubled by repeated subcutaneous injection of trypsin. The anti-enzymotic activity of the serum of two dogs which received 20 cc. of 4 per cent trypsin solution was increased after 24 hours in one instance to ten times its former strength. Some observers have assumed that the trypsin acts as an antigen and brings about the formation of a true antibody. Some have maintained that the anti-enzyme of normal serum is formed in response to the presence of enzyme within the body; Wiens (99) suggests that the enzyme of the leucocytes furnishes the stimulus to its formation. Colliner (12) thinks that trypsin absorbed from the pancreas, and K. Meyer (70) that autolytic and proteolytic enzymes derived from the tissues may act as antigen.

Specificity is one of the characters of antibodies formed when an antigen has entered the body. Eisner (17) found that sera which

varied in their anti-enzymotic action upon trypsin did not show corresponding variations when tested with labferment, pepsin, emulsin and lipase. Weil (105) cites evidence to show that anti-enzyme of the serum is not a specific antibody; human sera, he states, inhibit the vegetable protease papain in a constant ratio to their inhibition of trypsin. An observation of Pozerski (90) cited by Weil, is noteworthy in this connection; the serum of an animal immunized against papain contained a specific precipitin and an antibody which fixed complement but it possessed no increased ability to inhibit proteolysis caused by papain.

Increase of serum anti-enzyme following injection of trypsin is of little value as evidence of an immunity reaction, for similar increase is produced by a variety of apparently unrelated means. In animals which had received chloroform or phosphorus in quantity sufficient to produce profound intoxication Opie, Barker and Dochez (86) found an increase of proteolytic enzyme in the blood serum and in some instances complete disappearance of anti-enzyme. In animals which repeatedly received these substances in doses insufficient to cause death, there was a progressive increase of anti-enzyme. Braunstein and Kepnow (70) increased the antitrypsin of the blood by injecting an emulsion of tissue cells into the peritoneal cavity of rabbits. K. Meyer (70) found the antitrypsin of the blood increased in rabbits and dogs and also in patients after administration of thyroid tissue.

In view of the lack of evidence that the anti-enzyme of the serum is a true antibody attempts have been made to find in the serum some substance capable of inhibiting the action of proteolytic enzymes.

The antitryptic action of serum was attributed to lipid substances by Schwarz (94). He found that an emulsion of lipoids prepared from the organs of the horse by extraction with alcohol and ether and precipitation by acetone inhibited trypsin. By extraction with ether serum was deprived of its inhibiting action but addition of lipoids restored it. He suggests that serum antitrypsin is a combination of lipid with protein. Delezenne and Pozerski (14) had made the observation that serum treated with chloroform underwent autolysis. Sugimoto (96) found that the antitryptic action of egg white was removed by extraction with ether, petroleum ether, benzol and benzin, the effect of the last named being least. Addition of the lipid which had been removed, of lecithin, of lipid from brain and of lipid from liver to the egg white extracted with petroleum ether did not restore its inhibiting action and the lipoids themselves had little effect upon the tryptic digestion of casein.

Leucoprotease and trypsin were inhibited by Jobling and Petersen (34) with sodium soaps prepared from olive oil, croton oil, cod liver oil and linseed oil. Complete inhibition of tryptic digestion of casein was caused by 0.005 gram of linseed oil soap and partial inhibition was evident with much smaller quantities. The anti-enzymotic activity of the soap was inversely related to the saturation of its fatty acid and in proportion to its iodine value. Soaps of the saturated fatty acids such as sodium stearate or palmitate did not inhibit the action of the enzymes. Saturation of an unsaturated fatty acid with iodine removed the inhibiting activity of the soap.

Studies of the nature of the serum antitrypsin have been made by Jobling and Petersen (37). The antitryptic action of serum, lost by standing under ether during 4 days was partially restored when, after evaporation of the ether, sodium hydrate was added in sufficient quantity to give the ether treated serum a slightly alkaline reaction. The anti-enzyme is removed when the serum is acidified with hydrochloric acid (e. g., 5 cc. N/10 acid added to 20 cc. of serum from the dog) and filtered through kaolin; extraction of the kaolin with sodium alcoholate furnished a solution which inhibited the action of trypsin and had about one-tenth the strength of the untreated serum. Potassium iodide was found to diminish the antitryptic action of serum. When linseed oil soap was mixed with serum its inhibiting action, like that of serum alone, was completely removed by heating during thirty minutes at 70°C. From the evidence of these experiments the authors reach the conclusion that the enzyme-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids.

Slovzov and Xenophontava (95) found that the antitryptic substance of the serum can be extracted with chloroform but not with toluol. Since fatty acids have the same antitryptic action which is reduced by iodizing they reach the conclusion that the antitryptic action of the serum is caused by lipoids.

The lipoidal nature of antitrypsin is not accepted by Cobliner (12), Meyer (70) nor Teale and Bach (97), all of whom have found that extraction of dried sera with lipoid solvents such as chloroform, ether and petroleum ether does not remove its antitryptic power. Teale and Bach cite experiments to show, contrary to the opinion of Jobling and Petersen, that lipoids are more readily extracted from the dry than from wet serum and maintain that the lipoidal solvents destroy the anti-enzymotic activity of the serum only when they bring about coagulation of protein. Their observations concerning the inhibiting properties of soaps are at variance with those of Jobling and Petersen and they reach the conclusion that the serum antitrypsin is protein in nature.

**PROTEOLYTIC ENZYME OF MONONUCLEAR PHAGOCYTES (MACROPHAGES).** All mammals and many lower vertebrate species possess two types of cells capable of engulfing and digesting solid particles. One type is the polynuclear leucocyte (polymorphonuclear) with fine granulations, the neutrophile leucocyte of human blood which with inflammation leaves the blood vessels and forms the chief cellular element of acute inflammatory exudates. Its enzyme, leucoprotease, has been described. The second type of phagocytic cell is larger than the polynuclear leucocyte, it has a large nucleus which is round, oval or somewhat irregular in outline but never lobulated like the nucleus of the polynuclear leucocyte, and its cytoplasm contains no granules exhibiting a specific reaction to dyes. These cells have been designated macrophages by Metchnikoff and are the endothelial leucocytes of Mallory. During the later stages of acute inflammation when the inflammatory irritant has been overcome, the number of mononuclear phagocytes increases and these cells are actively engaged engulfing and digesting within their substance polynuclear leucocytes, red blood corpuscles and other cellular elements. When recovery occurs they have an important part in removing cellular elements from the site of inflammation. Moreover, they ingest certain parasitic microorganisms such as the malarial parasites and other protozoa, the tubercle bacillus, *B. leprae* and some other bacteria. Their phagocytic activity in typhoid fever is well known.

The proteolytic enzyme found associated with these phagocytic cells by Opie (81), unlike the enzyme of the polynuclear leucocytes, digests with greatest activity in the presence of an acid medium. In the inflammatory exudate obtained by injecting aleuronat into the pleural cavity of a dog, the number of polynuclear leucocytes decreases with the progress of the inflammatory reaction whereas the number of mononuclear cells exhibits a corresponding increase. A comparison between the character of the exuded cells during the first five days of the inflammatory reaction and the proteolysis which they cause when completely freed from serum has shown that there is coincident with decrease of polynuclear leucocytes, decreasing capacity to digest in the presence of an alkaline reaction and coincident with increase of mononuclear cells increasing power to digest in the presence of acid. The lymph nodes adjacent to the site of inflammation, for example, with sterile pleurisy caused by aleuronat, the substernal lymph nodes, afforded an opportunity to study the enzyme of the large mononuclear cells almost wholly free from leucoprotease. In these nodes large

mononuclear phagocytes occur in increasing number during the first five days of the inflammatory reaction and during this period there is increasing ability to cause proteolysis in the presence of acid but no digestion in an alkaline medium. The lymph nodes which drain the inflamed tissue and are crowded with mononuclear phagocytes exhibit much greater ability to cause proteolysis than distant lymph nodes, such as those of the mesentery, which contain few phagocytic cells. The enzyme of the mononuclear phagocytes which accumulate as the result of an inflammatory reaction has been designated lymphoprotease by Opie. It causes active digestion of protein in the presence of weakly acid reaction (0.2 per cent acetic acid) but is almost entirely inactive in the presence of a neutral or alkaline reaction. The enzyme is more susceptible to heat than leucoprotease being much impaired in activity by temperatures between 60° and 70°C. It is destroyed by higher temperatures. It is destroyed by drying after treatment with alcohol and ether. It resembles very closely the autolytic enzyme of parenchymatous tissues.

Two proteolytic enzymes have been found in experimental inflammatory exudates in the dog by Jobling and Strouse (49). One acts in an alkaline medium and is isolated by drying with alcohol and ether. The other acts in an acid medium and, unlike the alkaline-acting enzyme, is destroyed by heating to 70°C. during one-half hour. In hyperplastic lymph node in which the sinuses contained many large mononuclear cells, Longcope and Donhauser (61) found an enzyme which digested protein in the presence of weak acid.

The studies of Lord (62) and Nye (78) upon the hydrogen ion concentration favorable to the enzymes of a pneumonic exudate will be cited.

**EREPSIN IN LEUCOCYTES.** The occurrence within leucocytes of an enzyme with the character of the erepsin of Cohnheim is indicated by observations of Jobling and Strouse (49). Leucoprotease obtained after drying leucocytes in alcohol and ether failed to decompose proteoses and peptone to form amino-acids and no tryptophan was demonstrable. Fresh leucocytes split proteoses and peptone into these products in both acid and alkaline media but heat at 70°C. during one-half hour destroys this property. The leucocytes apparently contain an erepsin-like enzyme which acts upon products of the digestion caused by proteolytic enzymes. Parker and Franke (87) found that extracts of leucocytes of rabbit failed to cause any significant disintegration of protein but digested peptone to form amino-acid which was measured

by van Slyke's method. Similar digestion of a suspension of typhoid bacilli was attributed to the disintegration of "bacto-peptone." They believe that these leucocytes of rabbits contain erepsin but no protease.

Petersen and Short (89) found an increase of peptone-splitting enzyme in the blood serum immediately preceding and accompanying the crisis of lobar pneumonia.

**SERUM PROTEASE.** Enzymes similar in their action to those of the leucocytes may be found in the blood serum and after the coagulation of the blood enzymes are found attached to the fibrin which is formed.

When blood serum was treated with chloroform by Delezenne and Pozerski (90) the serum caused proteolysis of gelatin and casein. In the blood serum of the ox Hedin (27) found a weak proteolytic enzyme which acts in the presence of an alkaline medium and is present mainly in the globulin fraction of the serum. By treatment of blood serum with weak acid (0.2 per cent acetic acid), Opie and Barker (85) demonstrated the presence of a proteolytic enzyme; the globulin fraction of the serum obtained by half saturation with ammonium sulphate contained an enzyme which digested protein in neutral or alkaline media but failed to act in the presence of acid. This alkaline-acting enzyme resembles leucoprotease which does not digest in the presence of acid. It is probable that the serum contains two proteolytic enzymes.

With chloroform poisoning in dogs of such intensity that the liver undergoes necrosis and the coagulability of the blood is diminished, Opie, Barker and Dochez (86) found that the blood serum acquires increased ability to digest protein; this increased proteolysis is referable to an enzyme which digests with maximum activity in a weakly acid medium. The enzyme which digests in the presence of an alkaline medium is not increased.

Autolysis of serum after addition of chloroform has been used by Jobling and his co-workers in a series of studies on enzyme action (42) as a measure of serum protease. This method does not serve to identify the protease with either of those which have been recognized in the serum. It is noteworthy that antitrypsin which they have simultaneously measured restrains enzymes like trypsin and leucoprotease which act in an alkaline medium but is inactive in the presence of the acid reaction which is favorable to the acid-acting protease of the serum. Protease was present in serum (33) in greatest strength in guinea pigs and rabbits and was found in cat, ox and dog. In the normal dog it was found occasionally but was constantly found with distemper, pneumonia and inanition. In normal human serum they found little or

no protease. Serum protease cannot be identified with complement for it resists chloroform which destroys complement and is not destroyed by a temperature of 56°C.

Jobling and Petersen (38) found that the serum of guinea pig, rabbit and horse loses its antitryptic action when treated with kaolin, starch, agar or bacteria. They refer the toxic symptoms produced by sera so treated (anaphylatoxin) to toxic split products formed by the action of serum protease upon the proteins of the serum itself.

Several observers claim to have demonstrated in sensitized animals the presence of enzymes specific for the protein used as antigen. Jobling, Petersen and Eggstein (44) found the serum protease practically unaltered after the first injection of foreign protein; with acute anaphylactic shock, on the contrary, there was an "instantaneous mobilization" of non-specific protease and decrease in anti-enzyme. Intoxication they think is the result of cleavage products (peptones) liberated by enzyme action immediately after the introduction of the antigen. They do not regard the almost instantaneous occurrence of shock as a valid argument against this view.

The Abderhalden reaction demonstrated the presence in the serum of a proteolytic enzyme which Abderhalden has maintained has a specific action on placental tissue and is present in the serum only during pregnancy. This opinion has been much disputed and several of those who have investigated the subject have reached the conclusion that the serum itself is the source of the dialyzable products which are obtained, the placental tissue being undigested. Jobling, Eggstein and Petersen (33) maintain that protease of the serum becomes active because under the conditions of the method, the restraining influence of the anti-enzyme is removed. A full discussion of this subject is not desirable in this review.

In a series of papers Jobling, Petersen and Eggstein (43), (46), (47), (48), have described changes in serum enzymes and anti-enzymes following the injection of trypsin, kaolin, proteoses, peptones and bacteria into dogs. There was an increase of protease and usually an increase of antitrypsin which they regard as lipoid in nature. The amount of demonstrable enzyme bore no relation to variations in temperature or in count of leucocytes. They believe that a disturbance of ferment-anti-ferment balance in the serum may result in proteolysis of serum protein with liberation of toxic split products but they admit that these are not the sole agents of bacterial intoxication.

In the absence of profound fatal intoxication such as that caused by chloroform or phosphorus, anti-enzyme is present in the serum in sufficient quantity to restrain the action of serum protease. A complete absence of anti-enzyme, Jobling, Petersen and Eggstein state, is not essential for protease action provided some absorbing surface is present on which the relative balance of ferment-antiferment may be altered; "it seems probable that the protease action can take place in what might be termed local areas of antiferment deficiency, such as must occur at the point of contact of the serum and absorbing surface." Petersen (88) has discussed the speculative basis for recent attempts to find therapeutic use for agents which modify the enzyme and anti-enzyme of the serum.

No evidence that neutral fats, fatty acids or lipid bodies have a part in restraining the activity of serum protease has been found by Yamakawa (107). He measured proteolysis of serum by determining with the ninhydrin reaction the amount of dialyzable products formed from serum kept in a dialyzing thimble during 16 hours at 37°C. Serum of guinea pig undergoes proteolysis when treated with chloroform, methyl, ethyl and isobutyl alcohol and acetone but after removal of the chemical activator by vacuum, dialysis or extraction with indifferent chemicals (ether or petroleum ether) the original nonautolytic state does not return. Ethyl ether, petroleum ether, benzine and toluene neither activated nor paralyzed the serum protease. Addition of cholesterol, lecithin and neutral fats such as triolein and tripalmitin failed to influence autolysis.

**FIBRIN-PROTEASE.** Fibrin undergoes solution when allowed to stand in weakly acid, neutral or alkaline solution. The influence of varying temperatures upon solution and the formation of products of protein disintegration have shown that enzymes are concerned in the process. Studies of Rulot (92) furnish evidence that digestion of fibrin is caused by leucocytes imprisoned in the meshes of the fibrin. Proteolysis was measured by Kjeldahl determination of the nitrogen contained in decomposition products incoagulable by heat. To obtain fibrin free from leucocytes two methods were used. Clotting of drawn blood was prevented by addition of sodium chloride and the blood was centrifuged. Subsequent dilution caused the deposition of fibrin. In the second method the blood was made incoagulable by intravenous injection of propeptone; after centrifugalization, fibrin formation was brought about by a current of carbon dioxide. Pure fibrin obtained by these methods, Rulot found, was almost insoluble in physiological salt solu-

tion but underwent proteolysis when the leucocytes obtained by centrifugalization were added to it. In fibrin from the blood Barker (5) found a proteolytic enzyme which caused autolysis of fibrin and digested a protein substrate in presence of acid neutral and alkaline media.

In the fibrin of inflammatory exudates Barker (6) showed the presence of two enzymes. Leucoprotease was abundant in exuded fibrin in the early stage of acute inflammation produced by injection of turpentine into the pleural cavity of a dog and could be separated from the second enzyme which acts in acid by drying fibrin after treatment with alcohol and ether. After the second day of inflammation leucoprotease had disappeared but a proteolytic enzyme acting in an acid or neutral medium was abundant.

**INFLAMMATION.** At the site of an inflammatory reaction anti-enzyme of the blood passes with the exudate into the inflamed tissue or cavity and the leucocytes are surrounded by a fluid capable of inhibiting the proteolytic enzyme which they contain. The polynuclear leucocytes engulf solid particles and digest them within vacuoles in their substance. The anti-enzyme of the surrounding fluid serves to limit the activity of the enzyme to the site in which it is effective. Should the leucocyte be destroyed the enzyme which is set free can no longer cause proteolysis. With relatively mild inflammation such as that which occurs with lobar pneumonia or with serofibrinous pleurisy there is no solution of tissue and the part is ultimately restored to normal.

Study of enzymes has served to explain many of the phenomena of resolution. At the height of the inflammatory reaction the solid elements of the exudate are polynuclear leucocytes, red blood corpuscles, in small number, other cellular elements and fibrin. Leucoprotease appears to be chiefly concerned with the digestion of protein particles, such as bacteria engulfed by the polynuclear leucocytes. The mononuclear phagocytes which accumulate during the later stage of an inflammatory reaction which is proceeding toward recovery digest within their substance polynuclear leucocytes, red blood corpuscles and other cells. This phenomenon occurs not only at the site of inflammation but in the sinuses of the regional lymphatic nodes to which cellular elements as well as fluid are carried by way of the lymphatics. The enzyme of the large mononuclear phagocyte resembles the autolytic enzymes widely distributed in the parenchymatous tissues and is doubtless capable of causing autolysis and final disappearance of the macrophage under suitable conditions. It is often assumed that autolysis of the exudate is caused by the leucoprotease of polynuclear leucocytes but the correctness of this view has not been established.

When experimental pleurisy is produced by intrapleural injection of a sterile inflammatory irritant such as turpentine, serum accumulates and fibrin is deposited on the pleural surfaces. Accumulation of fluid reaches a maximum after 3 or 4 days and subsequently fluid rapidly subsides so that it has disappeared after 6 or 7 days. Reactions showing the presence of peptone and albuminoses were obtained by Opie (83) on and after the third day. Leucoprotease was present in the fibrin which was removed from the cavity during the first few days of inflammation and perhaps at this period had a part in causing its solution but later at a time when fluid had disappeared from the cavity this enzyme was no longer demonstrable and the fibrin which in diminishing amount was present in the pleural cavity during the next five or more days underwent autolysis only in the presence of weak acid. Final disappearance of fibrin is evidently brought about by an enzyme which has the characters of lympho-protease and resembles the autolytic enzymes of the tissues. It is difficult to determine what are the factors which bring this enzyme into action. It is noteworthy that the alkalinity of the exudate was less than that of the blood and diminished slightly with the progress of the inflammatory reaction.

When an inflammatory reaction is of such intensity that leucocytes accumulate in immense numbers and solution of tissue and of fibrin occurs, suppuration is established and resolution with restoration to normal is no longer possible. Opie (82) found that disintegration of polynuclear leucocytes in a purulent exudate sets free leucoprotease in quantity sufficient to completely overcome the anti-enzyme of the exuded serum. Both the whole pus and its fluid part separated by centrifugalization from the pus cells was now capable of causing proteolysis *in vitro*. The proteolytic activity of the exudate unrestrained by anti-enzyme explains the solvent action of pus for injured tissue and fibrin. An inflammatory irritant such as turpentine injected into the pleural cavity of the dog, where fluid rich in anti-enzyme readily accumulates, causes a serofibrinous inflammation which undergoes resolution with restoration of the cavity to normal. An equal quantity of the same irritant injected into the subcutaneous tissue where fluid accumulates with difficulty causes on the contrary very extensive suppuration with solution of tissue and healing by scar-formation.

In pus produced by pyogenic cocci, for example, with acute peritonitis, E. Müller and Kolaczek (75) found that the anti-enzyme was "saturated" by enzyme derived from leucocytes which had undergone disintegration; the fluid overlying the cells after centrifugalization exhibited proteolytic activity.

Since the normal spinal fluid, unlike other body fluids, contains neither enzyme nor anti-enzyme, Dochez (13) has studied the changes which occur with inflammation. With epidemic meningitis anti-enzyme may enter the spinal fluid but quickly leaves it. With the more severe inflammation caused by pneumococcus or streptococcus leucoprotease derived from disintegrating polynuclear leucocytes gives the fluid proteolytic activity which may itself act as an irritant and increase the severity of the disease.

In the exudates from acute arthritis, pleurisy, and wounds of war, E. Zunz (109) at La Panne in Belgium studied the relation of the anti-proteolytic property of inflammatory exudates to their alkaline reserve. The fluid was separated from the cells of the exudate and the quantity of this fluid necessary to inhibit a standard solution of trypsin was determined and designated the antitryptic index. The hydrogen ion concentration of the serum entirely freed from carbon dioxide was determined by the method of Marriott. The tryptic index and the alkaline reserve of serous or hemorrhagic exudates never exceeded and were almost always less than those of the blood serum of the same individual. The fluid from purulent exudates did not usually inhibit tryptic digestion and on the contrary digested coagulated egg white but occasionally this fluid had a slight antitryptic action. The alkaline reserve of seropurulent and purulent exudates was almost always less than that of serous exudates; increase of alkaline reserve was often parallel with an increase in the number of leucocytes and a diminution of the antitryptic index but the alkaline reserve tended to return to normal when the leucocytes diminished and the antitryptic index increased.

In serous exudates Zunz has found that diminution of complement is often parallel with diminution of antitryptic activity but this relation is not constant. In purulent exudates with complete or almost complete loss of antitryptic action complement has disappeared. Since the enzyme of leucocytes is present in excess the observation furnishes evidence, were more needed, to show that complement (microcytase) and proteolytic enzyme of leucocytes are not identical. Opie found that opsonin disappeared from an inflammatory exudate when it became purulent. It is probable that the loss of these bodies is referable to the action of unrestrained enzyme.

**PNEUMONIA.** The autolysis of the consolidated lung of lobar pneumonia was studied by Friedrich Müller (77); albumoses, leucin, tyrosin and other products of protein disintegration were formed at body temperature and nuclei disappeared as the result of decomposition of neu-

cleins. Flexner (22) found that autolysis took place much more quickly and perfectly in lungs in the stage of gray than of red hepatization and attributed the difference to the greater number of leucocytes with gray hepatization. Autolysis of the lung in instances of unresolved pneumonia was slow and incomplete.

By means of the serum plate method Lord and Nye (64) have studied the relation of enzyme to anti-enzyme at different periods of the disease. Sputum and exudate obtained at autopsy in the later stages of lobar pneumonia erode the serum plate. In the early stage of the disease proteolysis does not occur but if the cellular material obtained from the exudate is separated from the serum which accompanies it active digestion occurs. In this early period enzymes are inhibited by anti-enzymes. The author suggests that excessive impairment of circulation or excess of enzyme may result in disintegration of the pulmonary framework and abscess formation whereas excess of anti-enzyme may prevent resolution.

The hydrogen ion concentration favorable to the action of proteolytic enzymes contained in the cells derived from the pneumonic lung has been studied by Lord (62) and by Nye (78). They have used the serum plate, gelatin and peptone as substrate. The proteolytic enzymes are active with hydrogen ion concentrations between 7.3 and 6.7 and are inactive with higher, that is, more acid concentrations. Lord found an enzyme which splits peptone to form amino-acids; it acted with hydrogen ion concentrations between 8.0 and 4.8 but was most active between 6.3 and 5.2. He suggests that there is a gradual increase of the hydrogen ion concentration of the pneumonic exudate; the proteolytic enzyme or enzymes which act in a weakly alkaline and weakly acid medium are perhaps inhibited by the increasing hydrogen ion concentration but the peptone splitting enzyme is further activated when the hydrogen ion concentration of the exudate is increased to the range of its optimum activity.

In the blood serum of patients with lobar pneumonia Ascoli and Bez-zola (3), using the casein method of Fuld and Gross, found an increase of antitryptic activity in the earliest stage of the disease; this increase is maintained for a time and is followed by a decrease which occurs after the crisis. They have suggested that these changes are referable to a kinase which is derived from leucocytes and activates pancreatic trypsin; upon the basis of this speculation increase of antitrypsin is regarded as a reaction which follows the liberation of kinase by disintegration of leucocytes in the pneumonic exudate. Using the serum plate method to

test the strength of serum anti-enzyme for enzyme of pus cells Bittorf (8) found decrease of anti-enzymotic activity during the crisis followed by a considerable increase immediately after crisis and subsequently return to normal.

A study of *a*, the antitrypsin of the serum, its effect upon tryptic digestion being measured by Folin's method for nitrogen determination; of *b*, serum protease, measured by autolysis of serum after treatment with chloroform; and of *c*, non-coagulable nitrogen-containing substances of the blood, has been made by Jobling, Petersen and Eggstein (45). They find that the crisis of pneumonia is usually accompanied by 1, a decrease of the serum antiferment which before the crisis is increased; by 2, a mobilization of non-specific protease in the serum; and by 3, a decrease in the non-coagulable nitrogen and proteoses in the serum. They suggest that toxic split products of fibrin and of leucocytes rather than toxic products of the pneumococcus dominate the symptomatology of lobar pneumonia; crisis is perhaps the beginning of active autolysis depending upon an altered relation between ferment and antiferment so that with rapid autolysis toxic materials are destroyed.

In pneumonia that terminates by crisis or by lysis Petersen and Short (89) found that an increase of creptase (peptidase, erepsin) invariably precedes or accompanies the change of clinical symptoms associated with crisis or lysis, but in instances in which death occurs the creptase in the serum is usually less than that in the serum of a normal individual.

**LEUCEMIA.** The presence of incoagulable albumose-like substances in leucemic blood removed from the body after death was recognized by E. Ludwig (65). Erben (20) found that peptone and albumoses were not present in the blood when it was drawn but appeared after plasma and leucocytes separated from red corpuseles had stood in the incubator under aseptic conditions during three days. When the precipitate formed by treating leucocytes and plasma with alcohol was dried and extracted with glycerine the extract digested fibrin but lost this property when boiled. Normal blood and blood from cases of lymphatic leucemia did not undergo the change found with spleno-myelogenous leucemia. In cases of myelogenous leucemia, Schumm (93) demonstrated that products of protein disintegration were not present in the blood when it was removed from the body but appeared after the blood had been allowed to stand under chloroform. He attributes the change to a proteolytic enzyme.

E. Müller and Jochmann (71) showed that a serum plate was excavated by the blood of myelogenous leucemia when kept at 55°C. The blood of lymphatic leucemia did not produce these changes. Müller and Jochmann and Eppenstein (18) showed that the enzyme was in the cells of the blood and was inhibited by the serum.

Using the serum plate method, Jochmann and K. Ziegler (58) found that the bone marrow and spleen from cases of myeloid leucemia caused very active digestion; lymph nodes caused digestion in proportion to the myeloid transformation which they had undergone. Bone marrow of normal individuals caused active digestion of the serum plate, spleen caused slight digestion and lymph node none. Organs from cases of lymphatic leucemia and pseudo-leucemia, they state, cause no more digestion than those from normal individuals.

Studies have been made to determine if the large basophile cells of acute leucemia contain a proteolytic enzyme similar to that present in the myelocytes of myelogenous leucemia. In a case designated acute myeloid leucemia, K. Ziegler and Jochmann (108) found that half of the nucleated blood cells in the bone marrow and spleen were pure basophile cells or transitions between these and granular myelocytes; these tissues caused very active proteolysis of serum plates. Eppenstein (19) found no difference in enzymotic activity between the large lymphocytes of the blood from a case of acute lymphatic leucemia and the small lymphocytes from the chronic type of the disease. Müller and Jochmann (74) state that the blood cells in a case of acute lymphatic leucemia acted upon coagulated blood serum in the same manner as polynuclear leucocytes. Longcope and Donhauser (61) studied a case classified as acute lymphatic leucemia because the blood contained large mononuclear cells of the type of large lymphocytes with abundant faintly basophilic usually nongranular cytoplasm. Nevertheless a few neutrophilic myelocytes were found in the blood. The cells from the blood drawn during life and from lymphatic nodes obtained at autopsy caused proteolysis, measured by the Kjeldahl method, in an alkaline medium, and in this respect differed from the small lymphocytes of chronic lymphatic leucemia which caused no digestion and from the large mononuclear ("endothelial") cells of a hyperplastic lymph node which digested only in the presence of acid.

With myelogenous leucemia Jochmann and Kantorowicz (56) and Wiens (101) found no definite alteration of the anti-enzyme of the blood so that with a very high leucocytic count it may be normal. Wiens (102) suggests that enzyme set free by destruction of granular leucocytes

may stimulate formation of anti-enzyme so that the two are balanced. In fatal cases, he states, there may be a loss of anti-enzyme before death so that the serum may cause proteolysis.

**TUBERCULOSIS.** Caseous material from tuberculous lesions or the lung with tuberculous pneumonia did not undergo the active autolysis which Friedrich Müller (77) observed with lobar pneumonia. Heile (29) thought that the failure of caseous material to undergo absorption was due to absence of enzyme rather than to insolubility of caseous material. He found that the contents of a "cold abscess" did not digest fibrin but after injection of iodoform in glycerine polynuclear leucocytes appeared and the exudate now dissolved fibrin. E. Müller and Joehmann (71) found that fluid from a "cold abscess" unlike pus of acute inflammation did not excavate the surface of serum plates incubated at 55° to 60°C.

The presence in tuberculous tissue of a proteolytic enzyme digesting in a weakly acid medium was demonstrated by Opie and Barker (85). This enzyme resembled that of the large mononuclear phagocytes and digests protein more actively than the similar enzyme of parenchymatous organs such as the liver. The presence of tubercles within the liver increases the proteolytic activity of the tissue in neutral or acid media. These observations are in accord with the well-known histological characters of the tubercle which in great part consists of mononuclear cells capable of acting as phagocytes. The enzyme of tuberculous tissue is most abundant at a time when caseation is beginning but with advance of caseation its activity diminishes and with complete caseation total disappearance of enzyme seems to occur. Leucoprotease digesting protein in the presence of an alkaline reaction was found only when the tuberculous tissue was first formed and, examined microscopically, was found to contain some polynuclear leucocytes.

No autolysis of caseous material from lymph nodes first dried and ground and then suspended in water was found by Jobling and Petersen (36) unless the tuberculous nodes had become secondarily infected. When the lymph nodes were infected, autolysis occurred in the presence of alkaline and of acid reaction but was much more active in the latter instance. Affected areas in lungs with caseous pneumonia were freed as much as possible from less involved lung tissues, dried, ground, suspended in water and subjected to autolysis. The authors assume that the caseous material was not completely freed from inflammatory exudate. Autolysis was active in the presence of acid but absent when the medium was alkaline. With the purpose of removing inhibiting substances from the material under examination Jobling and Petersen

first treated some of it with acidified alcohol, centrifugalized, washed with alcohol and ether and dried. It then underwent autolysis in the presence of an alkaline reaction. The authors do not discuss the origin of this enzyme which, like leucoprotease, acts in an alkaline medium, and do not appear to have examined the tuberculous lung tissue to determine if it was secondarily infected with pyogenic microorganisms or contained polynuclear leucocytes.

From the bodies of tubercle bacilli Jobling and Petersen (35) have obtained an extract which inhibits the action of trypsin and of leucoprotease. An extract of tubercle bacilli made with ether and alcohol did not inhibit and an extract was prepared as follows: the ether-alcohol extract was dissolved in ether, precipitated with acetone, evaporated to dryness and saponified with alcoholic potash; the resulting soap was dissolved in water and repeatedly extracted with petroleum ether, treated with hydrochloric acid to liberate fatty acid, taken up with ether, washed with water and resaponified. An antitryptic agent was obtained. Though the soap obtained had a lower iodine value than soaps previously prepared from linseed, olive and cod liver oils, it had a greater inhibiting activity. This inhibiting action was lost when the soap was saturated with iodine. The same observers have extracted from caseous material of tuberculous lymph nodes and of the lung with caseous pneumonia, inhibiting agents with similar characters. They believe that these observations explain the failure of caseous material to undergo disintegration through the action of phagocytes or by autolysis but they do not assume that the inhibiting agent is wholly derived from the tubercle bacillus for they suggest that autolysis of the necrotic material of an anemic infarct is prevented by similar factors.

It is noteworthy that the observations which show that soaps of unsaturated fatty acids are anti-enzymotic agents have reference to trypsin and to leucoprotease. There has been no suggestion that trypsin is present and it is well known that polynuclear leucocytes in the absence of secondary infection are an inconspicuous constituent of tuberculous tissue and are not usually found in or about a caseous focus or an anemic infarct. Jobling and Petersen (41) have assembled evidence to show that potassium iodide which in moderate doses, they find, diminishes the antitryptic activity of the blood serum, hastens the softening of caseous material. A similar explanation is offered to explain the absorption of syphilitic gumma caused by potassium iodide. Nevertheless there are no observations to show that enzymes such as leucoprotease which are inhibited by the anti-enzyme of the blood serum are concerned in the absorption of the tubercle or of the gumma.

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# THE PHYSIOLOGY OF CREATINE AND CREATININE

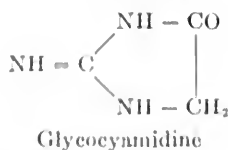
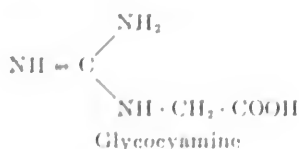
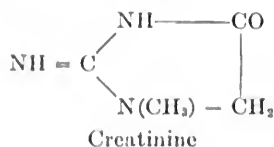
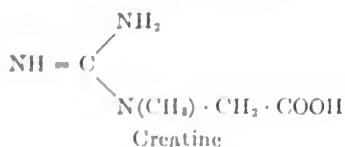
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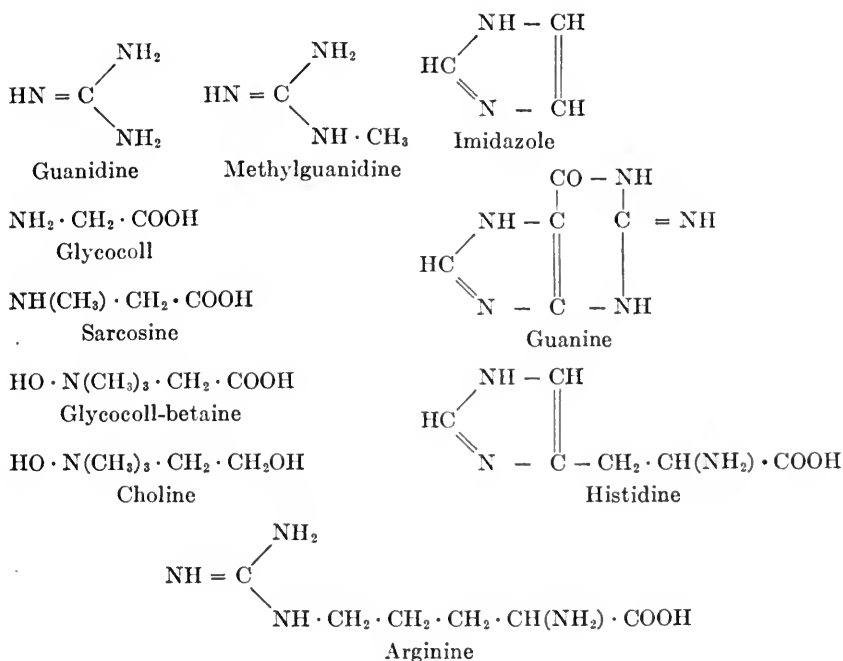
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*Creatine* is  $\alpha$ -methylguanidine-acetic acid, or methylglycoeyamine, and may accordingly be looked upon as a derivative primarily of acetic acid, or of glycocoll, sarcosine (methylglycocoll), guanidine, methylguanidine, or glycoeyamine (guanidine-acetic acid). From the standpoint of purely animal biochemistry one of its most distinguishing structural characteristics is its N-methyl radicle, which brings it into relation with the betaines and with choline. Its guanidine group, on the other hand, suggests a connection with arginine, the only known derivative of guanidine among the products of protein hydrolysis.

*Creatinine*, 2-imido-5-keto-3-methyl-tetrahydroimidazole, or methylglycoeyamidine, is the internal anhydride of creatine, to which therefore it is related in the same way as glycoeyamidine to glycoeyamine. The ring resulting from the loss of a water molecule is built upon the imidazole (or glyoxaline) nucleus, so that creatinine possesses, besides the relations dependent upon its derivation from creatine, others with such biochemically important imidazole derivatives as histidine and the purines. Among the latter guanine, 2-amino-6-oxypurine, may claim a two-fold connection with creatinine, for in addition to the imidazole nucleus common to every purine its structure includes, as part of its pyrimidine complex, a guanidine residue.

The structural interrelationships of the compounds mentioned are made obvious in the accompanying formulae:





Of the chemical properties of creatinine and creatine two are of special importance from the point of view of the biochemist.

The first is the power of creatinine to react with alkaline sodium picrate with the formation of an intensely red reduction product, probably sodium picramate. Upon this reaction, first described by Jaffé (105), Folin (61) based a colorimetric method for the determination of creatinine, from the introduction of which in 1904 all sure progress in the quantitative study of creatine-creatinine metabolism can be dated. Folin (61), (64) further utilized the reaction in a method for the indirect determination of *creatine*, involving its conversion into creatinine by heating under suitable conditions (see below) with an acid. In their original form these methods were adapted only to the determination of relatively considerable quantities of "total" or pre-formed creatinine, but they have been variously modified, by Folin himself and by others, so as to be applicable to the analysis of very small volumes or very great dilutions of urine (184), (67), or to the determination of creatine and creatinine in muscle (160), (184), (68), (12), (145), (107), (14), (15), blood (184), (68), (148), (214), (51), (79) (71), tissues generally (184), (68) and milk (68). The original pro-

cedure for the conversion of creatine to creatinine has also received various modifications (169), (67), (27), (13), (83). While the application of these methods is by no means free from possible errors and fallacies, they have constituted, as a whole, one of the most useful and convenient weapons of biochemical research ever forged.

The second important property of creatine and creatinine is the readiness of each to undergo, under appropriate conditions, a transformation into the other. In alkaline media the transformation is mutual, reversible and fairly rapid. Thus in N/10 NaOH a 0.38 per cent creatine solution reaches equilibrium in about 2 days at 26°, and contains then about 0.1 per cent of creatinine (82). The determination of the equilibrium constant ( $K = \frac{[\text{creatinine}]}{[\text{creatine}]}$ ) is rendered difficult by the fact that alkalis bring about also a gradual decomposition of creatinine; but it has been estimated indirectly to have approximately the value 2.12 (82). In a pure aqueous solution at 36° equilibrium is attained only after 11 months (147), but in 21½ hours at 98° (83); the molecular ratio of creatine to creatinine is then only 0.4 (82). In acid solutions the equilibrium point is so far on the side of creatinine—a fact to be explained by the greater basicity of that substance (82)—that the reaction is practically irreversible; in other words acids convert creatine completely into creatinine, but leave the latter unaffected. The velocity of the conversion, for any given concentration of creatine, depends on the temperature and on the concentration of hydrogen ions. In normal HCl the change, for small concentrations of creatine, is complete in 15 days at 26°, and within 24 hours at 60° (82), (83); in N/2 HCl it takes about 3 hours at 98° (64), and 15 minutes at 117° (25).

**BIOLOGICAL DISTRIBUTION OF CREATINE AND CREATININE.** Creatine and creatinine are known with certainty only as products of the metabolism of vertebrates. Reports of the presence of creatinine in the culture media of bacteria (10), (75), (60), (178), (179), (137), or, along with creatine, in the tissues of *Abalone*, a mollusc (8), are based as yet only upon imperfectly specific color reactions.

*Creatinine* is a constant constituent of the urine of mammals, occupying as a rule a place among the organic nitrogenous catabolites second in quantitative importance only to that of urea. In the urine of birds it is relatively less abundant, being replaced there largely, although apparently not entirely, by creatine (156), (196). Reptiles appear in this respect to resemble birds (124). The meager data available regarding fishes (44), (45) seem to put elasmobranchs with the mammals,

teleosts with the birds. The position of the amphibia is unknown; van der Heyde (89) could find neither creatinine nor creatine in the urine of hibernating frogs. Most adult mammals—ruminants forming, it would seem, an exception (153)—excrete under normal circumstances either no creatine at all or only irregular traces.

Creatinine is believed to occur also, although in minute quantities only, in the tissues and fluids of the body. Typical examples of the concentrations which have been reported will be found in table 1. The data are derived entirely from micro-analytical applications of the Folin method, and are undoubtedly subject to considerable error (102), (214), (79). In the particular case of blood Behre and Benedict (20) have indeed recently produced evidence tending to show that the error of the determination greatly exceeds any possible quantity of creatinine that might be present. Their findings should probably be regarded as still requiring confirmation; but if they should prove to be correct for blood, grave doubt will be thrown upon the supposed concentration, and even the existence, of creatinine in other tissues also. For the present the creatinine figures of table 1 will be assumed to possess at least a relative value; but it must be recognized that any conclusion of which they form an essential premise lies under some suspicion.

*Creatine* is a characteristic component of certain special tissues, and is possibly, as indicated again by the quantitative data of table 1, not altogether lacking in any. Its presence has been unequivocally demonstrated, either by actual isolation in substance or by production, after dehydration, of the characteristic creatinine zinc chloride, in the cases of striated muscle of the vertebrates generally (including mammals, birds, amphibians, fishes, and the lamprey), of mammalian heart muscle, of brain (21), and of the blood as a whole (20). It has been shown, by the same criterion, that blood serum contains certainly either creatine or creatinine or both (121). For the other tissues named in table 1 (including, as far as I have been able to discover, the smooth muscles of vertebrates) the evidence for the occurrence of creatine is limited to the positive outcome of the Jaffé test after treatment of the tissue or its extract with acids. Where this test indicates really notable quantities of creatine, as with the testis, it is perhaps justifiable to assume that actual creatine could be obtained from the tissue if anyone took the trouble to seek it; but where its reported concentration is as low as it is with smooth muscle and with the majority of the non-muscular tissues, a certain amount of scepticism regarding its presence is excusable.

TABLE 1  
*Creatine and creatinine content of tissues*  
 (Mgm. per 100 gm.)

TISSUE	ANIMAL	CREA- TINE	CREATININE	AUTHORITY
Striated muscle.....	Man (adult)	393-430	10.0-11.5	(184)
Striated muscle.....	Man ( $1\frac{3}{4}$ years)	331		(46)
Striated muscle.....	Man (at birth)	190		(168)
Striated muscle.....	Rabbit	494-540	3.4- 9.2	(140)
Striated muscle (white).....	Rabbit	404-578		(159)
Striated muscle (red).....	Rabbit	277-380		(159)
Striated muscle.....	Rabbit (adult)	430		(133)
Striated muscle.....	Rabbit (39 days)	390		(133)
Striated muscle.....	Rabbit (19 days)	316		(133)
Striated muscle.....	Rabbit (9 days)	228		(133)
Striated muscle.....	Rabbit (7 days)	191		(133)
Striated muscle.....	Rabbit (fetal)	Trace		(133)
Striated muscle.....	Cat	421-580	2.3- 8.0	(69) (70)
Striated muscle.....	Pig	364-410		(21)
Striated muscle.....	Ox	421-530	10.3-12.7	(21) (184)
Striated muscle (fetal).....	Ox	26-290		(21)
Striated muscle.....	Sheep	406-419		(93)
Striated muscle.....	Horse	376-395		(93)
Striated muscle.....	Dog	408-488	5.0-15.6	(69) (184)
Striated muscle.....	Guinea pig	370		(133)
Striated muscle.....	Rat	470		(140)
Striated muscle (breast).....	Hen	408-481	5.0- 5.3	(35) (140)
Striated muscle (leg).....	Hen	348-368	1.6- 1.9	(35) (140)
Striated muscle.....	Frog	364-399		(175)
Striated muscle.....	Turtle	236-339		(69)
Striated muscle.....	Skate	280		(133)
Striated muscle.....	Cod	350		(133)
Striated muscle.....	Carp	421	77	(151)
Striated muscle.....	Shark	655	134	(151)
Striated muscle.....	<i>Phycis</i>	295-313		(35)
Striated muscle.....	Lamprey	290		(133)
Cardiac muscle.....	Ox	240-255		(21)
Cardiac muscle (fetal).....	Ox	31-54		(21)
Cardiac muscle.....	Dog	210-327		(69)
Cardiac muscle.....	Cat	221-333		(69)
Cardiac muscle.....	Rabbit	186-291		(69)
Cardiac muscle.....	Sheep	208-339		(69)
Cardiac muscle.....	Hen	166-190		(69)
Cardiac muscle.....	Turtle	70-109		(69)

TABLE 1—*Concluded*

TISSUE	ANIMAL	CREA- TINE	CREATININE	AUTHORITY
Smooth muscle				
Uterus.....	Cow	35-50		(21)
Uterus (at term).....	Cow	98-104		(21)
Uterus.....	Human	52		(21)
Uterus (at term).....	Human	89		(21)
Uterus (post-partum).....	Human	51		(21)
Colon.....	Rabbit	36		(21)
Small intestine.....	Rabbit	27		(21)
Gizzard.....	Goose	70		(156)
Bladder.....	Ox	99-130		(35)
Brain.....	Dog	108		(107)
Brain.....	Ox	103		(107)
Testis.....	Bull	195		(14)
Testis.....	Dog	181		(107)
Testis.....	Sheep	209-215		(14)
Liver.....	Dog	21-28	2.8	(14) (184)
Pancreas.....	Ox	14-23		(21)
Kidney.....	Ox	14-20		(21)
Spleen.....	Dog	15-23	4.0 (Cat)	(21) (140)
Thyroid.....	Calf	13	1.1	(21) (140)
Thymus.....	Calf	11		(21)
Blood.....	Man	2.1-4.9	0.7- 1.3	(103)
Blood.....	Hen	11	0.1	(69a)
Milk.....	Cow	2.0-2.6	1.0- 1.5	(53)
Milk.....	Human	1.9-3.9	1.0- 1.6	(54)

Table 1 includes not by any means all the data to be found in the literature upon the creatine content of tissues, but such a selection as may illustrate the chief peculiarities of its distribution. It will be seen that while noteworthy concentrations of creatine are to be found in the brain, the testes and the gravid uterus, with quantities greater still in the myocardium, no other tissue contains so high a percentage as voluntary muscle. Taking into account the average relative mass of the different organs, Bürger (32) has calculated that about 98 per cent of all the creatine in the human body is carried by the muscles, and about three fourths of the small remainder by the brain.

The creatine concentration of the muscles of a given species, among individuals chosen at random, varies within limits that are frequently

rather wide; but there are cases like that of the rabbit (140), (164) where, as the consequence perhaps of uniform conditions of feeding and environment, the muscle creatine has been found to be very nearly a constant. The variability, such as it is, does not obscure the fact that there is a certain average concentration of creatine fairly characteristic for each species.

The figures reproduced furnish evidence for some other interesting generalizations. The creatine content of muscle is not the same at all ages, but rises steadily during fetal and post natal growth till it attains the maximum characteristic of the adult. There is more creatine in the quickly contracting pale muscle than in the slowly contracting red, a difference exhibited alike by mammal and by bird. The muscle creatine of warm-blooded animals is generally speaking higher than that of the more sluggish cold-blooded, although some of the data for fish supply striking exceptions. Smooth muscle has a very much lower concentration of creatine than striped; and the gravid uterus a higher one than the non-gravid. All these facts point in one direction. They indicate very strongly that creatine is a substance with a useful function, a function in some way connected with that capacity for rapid and powerful contraction which is the most important property of striated vertebrate muscle.

CREATININE AS A CATABOLITE. Whatever the rôle of creatine may be, there is no doubt that creatinine is a mere waste product. This is demonstrated by the fact that when it is administered to animals, whether parenterally (202), (143), (176), (126) or by mouth (93), (64), (94), (216), (72), (202), (176), (172), (74), the greater part, up to 80 per cent or more, is promptly excreted. The missing 20 per cent or so has in some instances been traced to the muscles, where it has doubtless undergone the reversible transformation into creatine (143). None of it is ever converted within the body into urea or ammonia (64), (70), (172) or other known product of further degradation. Not only therefore is creatinine a waste material, but it is a terminal and not merely an intermediate product of catabolism.

The daily output of this waste product, upon a diet free from either creatine or creatinine itself, is for each individual a practically constant quantity, entirely independent of the amount of protein in the food, or of the total nitrogen catabolism, or of variations in the volume of the urine. This striking and fundamental law of metabolism, the discovery of which constitutes the real starting point of modern advance in the physiology of creatinine, was first announced in 1905 by Folin (62), and

has been fully confirmed by many later investigators (93), (43), (109), (181), (177). The "constancy" of the daily output is of course not absolute. It would appear from data scattered throughout the literature that the day to day variations may even amount to 20 or 25 per cent; but they do not often exceed 10 per cent, and are often for long periods together very much less. In any case they bear no relation to nitrogen output or diuresis.

According to Shaffer (182) the rate of elimination is uniform not only from day to day but even from hour to hour. The data of Klercker (109), Hoogenhuyze and Verploegh (95), (94), and Neuwirth (150) show no such regularity; and Schulz (177), who collected his own urine in two-hour periods for many consecutive days, found periodic variations with definite maxima, persistent even during fasting, at 9-11 a.m., 3-5 p.m. and 9-11 p.m. Schulz's data show also that the average of the night hours is lower than the average for the day. His observations were limited to one individual and it is not certain that they can be generalized. The lower rate of elimination during the night has been observed by Powis and Raper (162) and by Campbell and Webster (36), and is generally evident in the extensive data of Hoogenhuyze and Verploegh.

While the daily creatinine output is approximately a constant for the individual, it varies decidedly from one individual to another. Folin (62) recognized that the variations stood in some relation to the weight of the subject, and Shaffer (182) introduced the practice of expressing this relation by means of the "creatinine coefficient"—the number of milligrams of creatinine (or creatinine nitrogen) excreted daily per kilogram of body weight. This coefficient, expressed in terms of creatinine, varies in "strictly normal" human adults of the male sex from 18 to 32 (182); though possibly the lower limit of normality should be reduced to 15. In normal women the figures have the decidedly lower range of 9 to 26, averaging only 15.6 (203); it is probable, as Shaffer thought, that this is not an effect of sex in itself, for individual women whose muscles have been developed by gymnastic training have coefficients comparable with those of men (203). Infants and children have lower coefficients still; 6.7 to 10 at 10 to 14 days (9), and 9 to 17 at 5 to 13 years (118). The considerable range of the figures in every group shows that the relation between creatinine output and total body weight is not one of strict proportionality. What other factors intervene will be considered presently.

**METABOLIC SIGNIFICANCE OF URINARY CREATININE.** The constancy of the creatinine output and its utter lack of relation to the total protein metabolism were interpreted by Folin (63) as indicating that creatinine is the product of a special quantitatively unvarying form of protein metabolism, described as constituting an "essential part of the activity which distinguishes living cells from dead ones," named "*tissue* or *endogenous* metabolism," and sharply differentiated from the variable "*exogenous*" metabolism of the food protein. Apparently this special type of metabolism was conceived of as taking place in all the living protoplasm of the body. Differences in the creatinine coefficient were ascribed to differences in adiposity, that is, to differences in the relative amount of metabolically inert substance in the body; and the conclusion was implied, if not expressly drawn, that the creatinine output depends on the mass of active protoplasmic tissue. In his latest exposition of his views (70) Folin, indeed, states positively that he regards creatinine as an "index or measure of the total normal tissue metabolism."

In its main point, the idea of creatinine as the product of a special tissue metabolism and as having no relation to the catabolism of food protein, this conception has met with practically universal acceptance. In its details it has been variously modified. Shaffer (182) showed that there is a close parallelism between the creatinine coefficient of the individual and his muscular development, strength, or "efficiency." He suggested therefore that creatinine "is derived from, and an index of, not the total tissue or endogenous catabolism, but of one special process of this catabolism," taking place "largely if not wholly in the muscles," upon the intensity of which depends "the muscular efficiency of the individual." Similar views were expressed by Spriggs (186). There is of course an obvious probability that the production of creatinine should take place mainly in the tissue which is richest in creatine; but Shaffer's conclusions were reached independently of this consideration.

There are certain developments of Shaffer's view in which the direct derivation of creatinine from muscle creatine is taken as granted or proved. The first of these is the hypothesis of Pekelharing and Hoogenbuijze (159), which attempts to define more precisely than Shaffer did the relation between creatine or creatinine and muscular "tone." On this hypothesis the chemical transformations involved in the maintenance of tonus are considered to be entirely different from those associated with the rapid tetanic contractions of voluntary movement; while

the latter consist preëminently in the oxidation of carbohydrate, the former are thought to include changes of nitrogenous material and in particular the formation of creatine. The output of creatinine is therefore determined by the state of tone of the muscular system.

This theory was supported by a great deal of experimental evidence tending to show that muscles in a state of "tonic" contraction, brought about in a great variety of ways, gained in creatine, and that sustained contraction or "tonus" such as is involved in the maintenance of the Prussian military posture is accompanied, in contrast with ordinary muscular effort, by an increased output of creatinine (161). It may be questioned whether all the forms of continuous contraction studied by Pekelharing and Hoogenhuyze were examples of exaggerated "tonus" in the strict sense of the term. In any case Brown and Cathcart (31) obtained comparable increases of creatine in frog muscles stimulated to contract in the ordinary way, and Schulz (177) failed to confirm the alleged special effect upon creatinine output of sustained contraction in man. It is more than doubtful therefore whether the distinction drawn between the chemical mechanism of tonus and that of voluntary contractions can be taken as established. Some relation, we have seen already, probably does exist between the functional efficiency of striped muscle (one factor in the maintenance of which is tonus) and its creatine content; but as far as the evidence goes it is just as likely that tonus is dependent on creatine, as that creatine and creatinine are products of tonus.

The theory just discussed has this in common with that of Shaffer that it makes urinary creatinine an index of muscular tone. According to Myers and Fine (140) it is simply an index of muscle creatine. In a manner this is a synthesis of the two precedent hypotheses, which somewhat to its advantage leaves open the question of the significance of the creatine. Myers and Fine observed that there is an approximate proportionality between the creatine concentration in the muscles (or entire body) of an animal and its creatinine coefficient. Thus the average creatinine coefficients of the rabbit, man and dog are respectively 38.4, 24.2 and 22.5; the average percentages of creatine in the muscles of these animals are, in the same order, 0.52, 0.39 and 0.37. The coefficients stand in the ratio 1.7 : 1.07 : 1.0; the creatine concentrations in the ratio 1.4 : 1.05 : 1.0. Comparison of different individuals within a single species leads to a similar result: for 5 rabbits, with coefficients between 36 and 37, the average concentration of creatine in the entire body was 0.170 per cent; for 5 others with co-

efficients of 38 to 41 (average 40.3), the average creatine percentage was 0.193. These parallelisms strongly suggest that there is a constant relation between the total amount of creatine in the body (or, what for all practical purposes is the same, in the muscles) and the amount of creatinine in the daily urine. Direct observations upon a series of 12 rabbits showed a total body creatine ranging from 4.317 to 0.947 grams with a creatinine output of 99.9 to 19.2 mgm., while the ratio of the two lay between the comparatively narrow limits of 43.2 and 53.3—in five cases out of the twelve between 44.4 and 45. It would appear therefore that animals with a high creatinine elimination, whatever may be their creatinine coefficient, do actually have a correspondingly high body content of creatine. All these correlations Myers and Fine interpret as indicating that urinary creatinine and muscle creatine are intimately related in metabolism, that the former takes origin either from the latter or from some common precursor, and that the relative constancy of muscle creatine in the normal animal affords an immediate explanation of the uniform rate at which creatinine is eliminated in the urine.

There are many flaws in this argument, which has been severely criticised by Benedict and Osterberg (28); but the case which Myers and Fine make out is none the less a rather strong one. The apparent demonstration of a relation, quite independent of body weight and creatinine coefficient, between urinary creatinine and total body creatine is especially to the point. The thesis has the merit of simplicity, and there are no known facts utterly incompatible with it. It has apparently been adopted by Shaffer (184) as consistent with, and complementary to, his own theory. It leaves of course unexplained the cause and meaning of the relative constancy, in individual and species, of muscle creatine.

Since all but 2 per cent or so of the body's creatine is in the muscles, it is an obvious corollary of the above theory that, so long as the muscles are in a normal state (i.e., possess a normal creatine content), urinary creatinine is determined by muscle mass, and that conversely, if once we ascertained for any species the factor connecting them, the mass of an animal's muscles could be calculated from its creatinine output. This deduction was made by Myers and Fine themselves, but, wisely perhaps, they attempted no practical application of it. Bürger (32) has recently ventured a calculation which would indicate that 1 gram of urinary creatinine per day corresponds in man to 22.9 kgm. of normal muscle. He is on surer ground when he points out that, if creatinine is

an index of muscle creatine, the creatinine coefficient becomes under physiological conditions an index of, and indeed proportional to, the participation of the muscles in the body weight. The fact that clinically, as he shows, the coefficient, in the absence of disturbing factors, does vary in a regular way with the apparent predominance of the muscles, lends a certain amount of support to the theory.

ORIGIN OF CREATININE. It was impossible to discuss the metabolic significance of the urinary creatinine without touching upon its origin; but in some respects this may be treated as an independent problem.

It was at one time believed that creatine was readily and largely transformed in the body into creatinine, and the direct derivation of the latter from the former was therefore practically taken for granted. When Folin in 1906 (64) undertook, by the aid of his newly developed colorimetric method, a study of the fate of ingested creatine, these beliefs were for the first time seriously disturbed. The essential data of three of Folin's experiments are reproduced in table 2. (In this table all reported figures for creatine have been converted into terms of the anhydrous substance as such, and a calculation has been made of the amount not accounted for in the urine as either creatine or creatinine; this is reported under the heading of "creatine retained.") The results, as illustrated, may be stated thus: *a*, creatine administration led to no increase of creatinine output; *b*, with doses of about 5 grams a certain amount of creatine was usually excreted unaltered, the proportion so eliminated being greater upon a high than a low protein diet; *c*, with small doses (1 gram or thereabout) no creatine at all reappeared; *d*, in no case was there any increase in urea or, it may be said, in ammonia, or (except once) in the undetermined nitrogen of the urine.

Such results, entirely at variance with prevailing notions, led Folin to conclude that the organism does not possess the power of converting creatine into creatinine, that these two substances are quite independent of each other in metabolism, and that creatine is not, like creatinine, a waste product, but a food.

The observation that creatine introduced from without may be partly excreted unchanged, but is usually to a large extent or even altogether retained within the organism, has been repeatedly confirmed, for animals as well as men, and not only for oral (109), (94), (216), (122), (161a), (72), (202), (118), (176), (144), (172), (74), (76), but for parenteral administration (120), (160), (202), (143), (176), (126). That none of it is ever converted into creatinine has been shown to be an error (94), (160), (202), (72), (143), (176), (144), (172), (126). If

the dose given is large enough, the increased output of creatinine is unmistakable. This is strikingly shown by two experiments of Rose and Dimmitt (172), one of which is reproduced in table 2; it will be seen that in this particular case even 1 gram of creatine produced a slight increase of creatinine, while with 20 grams the output rose to 36 per cent above the endogenous level. It may be said that in the

TABLE 2  
*Fate of ingested creatine*

CREATINE GIVEN	TOTAL N	UREA N	CREATI- NINE	EXTRA CREATI- NINE	CREA- TINE EX- CRETED	CREA- TINE RE- TAINED	AUTHOR
<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	
0.97	5.49	4.18	1.33				Folin
	5.39	3.91	1.33	0	0	0.97	
	3.99	2.80	1.34				
4.4 (1 dose)	3.89	2.39	1.83				Folin
	3.67	1.83	1.69	0	0.84	3.56	
	3.76	2.30	1.80				
4.4 (3 doses)	19.88	16.44	1.53				Folin
	21.70	17.69	1.53	0	2.34	2.06	
	20.16	16.96	1.37				
	11.05		1.35				Rose and Dimmitt
	10.92		1.29				
	10.42		1.35				
1	10.41		1.45	0.10	0.09	0.79	
2	10.58		1.51	0.16	0.13	1.68	
5	11.48		1.59	0.24	1.81	2.91	
10	12.83		1.61	0.26	5.81	3.88	
10	13.78		1.69	0.34	5.90	3.70	
20	16.86		1.83	0.48	15.10	4.34	
	11.64		1.67	0.32	0.41		
	9.87		1.61	0.26			
	11.07		1.61	0.26			
	10.40		1.52	0.17			

other experiment of Rose and Dimmitt, where urea determinations were made, there was no evidence, even after 20 grams of creatine had been given, that any of it was converted in the body into urea or ammonia. On this point Folin's conclusion is fully substantiated. Creatine therefore is not subject in metabolism to any catabolic "destruction," other than by conversion into creatinine.

The demonstration that exogenous creatine is partly transformed into creatinine may not by itself prove that endogenous creatinine has its origin in body creatine, but it disposes of the only experimental evidence irreconcilable with such an idea. The extent of the transformation is admittedly small—roughly 5 per cent of the ingested creatine; but it may be questioned if there is any real reason for expecting it to be greater. The amount of creatinine produced will depend in the first place on the active mass of the creatine at the place where dehydration occurs. There is no way of telling what that “active mass” is; but let it be assumed that it is constituted by the total creatine of the muscles, that the muscles are the sole seat of the reaction, and that accordingly only that portion of the ingested creatine which is incorporated in the muscle substance can contribute to the formation of creatinine. When creatine is introduced from without the muscles are offered an excess of a special tissue component, of which they already as a rule have a sufficient supply; small doses they may be able to absorb completely, but of a large dose much will be rejected, will on the assumption made never enter the metabolic circle, and will accordingly be excreted unchanged. Now the total creatine of the muscles amounts, for an average man, to about 112 grams (32). If that quantity be increased by the addition of 1 gram of ingested and retained creatine, it need not surprise us that the effect upon the creatinine output is imperceptible. In the second of Rose and Dimmitt's experiments (not reproduced in the table) the amount retained out of 20 grams was only 5.7, which is the largest retention ever recorded for a single day's experiment. If all of this were added to 112 grams it would mean an increase of about 5 per cent. The increase of urinary creatinine actually observed was about 0.3 gram; small as it is, it represents an excess of 18.5 per cent over the endogenous output, and is therefore more than one is forced to expect. The argument, as stated, involves several unverifiable assumptions, and in a quantitative sense deals with the crudest approximations; but in its main outline it is probably perfectly sound.

The opinion that creatinine is derived from muscle creatine is supported by the observation (184), (145), (70) that muscle contains more free creatinine than any other tissue, and more than the blood which passes through it (see table 1); as well as by the accelerated and probably enzymatic transformation of creatine into creatinine, without loss of total creatinine, which takes place in muscles or muscle extracts after death (147), (84). The first of these points has its force rather

weakened by recent evidence tending to show that blood contains no creatinine at all (20), and the second by the fact that the muscles of birds, which are said to excrete creatine in place of creatinine, show the same changes upon autolysis as those of mammals (147). It may be admitted, therefore, that no single point in the evidence, not even the partial transformation in the body of creatine into creatinine, demonstrates in a clear cut fashion the origin in normal metabolism of creatinine from creatine. Yet, when the entire body of evidence, including the apparent relations between muscle mass or muscle creatine and urinary creatinine or creatinine coefficient, is passed in review, and when it is considered that the actual ability of the body to convert ingested creatine into creatinine is no longer in doubt, it is easy to understand why the old doctrine of the origin of creatinine from creatine meets again today with practically universal acceptance.

One qualification, however, is usually present in the minds of those who subscribe to that doctrine (140), (184). The "creatine" of the living muscles may not be free creatine. There is indeed some difficulty in believing that it is free, for it is present in a concentration very much higher than that in the blood and yet the muscles are able to take up still more creatine from the circulation (70). It would seem therefore that the creatine must be held in the muscles by some special attractive force, either chemical or physical. This leads to the idea of a "creatine-containing complex," and it is possibly some such complex, rather than creatine itself, which is to be regarded as the actual precursor of creatinine. Whether in metabolism this creatine-complex, if it exists, is to be thought of as first yielding creatine, which thereupon is transformed into creatinine, or whether it yields the latter directly, is not very clear.

The second of these alternatives corresponds with the hypothesis of Folin and Denis (70), who still maintain the essential independence of creatine and creatinine; but in their view the creatine-complex is nothing short of the living protoplasm itself. The creatine of muscle, they think, is entirely a post-mortem product. The metabolism of living protoplasm gives rise directly and solely to creatinine; but at death this same protoplasm, in muscle at least, breaks down in such a way as now to liberate creatine. The chief difficulty in accepting this hypothesis is that it implies that the "death" of protoplasm is practically an instantaneous phenomenon; for no matter how suddenly an animal is killed, how rapidly or at how low a temperature its muscles are submitted to extraction, one obtains practically the maximum yield of creatine (158)

If the creatine of living muscles is in some sort of combination it must be a combination of the loosest possible kind. The case is not very different from that of the amino acids. These also accumulate in the tissues from lower concentrations in the blood (210); but it has not been suggested that they therefore become an integral part of the protoplasm, or that they are not themselves the direct precursors of urea. Although doubtless held by the tissues in some kind of loose molecular combination the amino acids behave in metabolism as if perfectly free. Probably the creatine of muscle may be regarded as doing the same.

THE INFLUENCE OF MUSCULAR WORK UPON CREATINE-CREATININE METABOLISM. The *total* output of creatinine for the day is independent not only of the quantity of protein in the diet, but also of the amount of ordinary muscular work performed (93), (181). This appears to be true even when the diet is of inadequate energy value (93). Whether it holds during a complete fast appears to be somewhat uncertain; the recent experiments of Schulz (177) throw some doubt on the conclusion drawn (from a single experiment) by Hoogenhuyze and Verploegh (93) that exercise during fasting causes a considerable rise in the daily excretion of creatinine.

It does not follow that muscular contractions have no effect at all upon creatine metabolism. An effect becomes obvious enough when the output of creatinine is measured not simply day by day, but at intervals as short as two hours. It is then found (177) that the output of any work period is decidedly and invariably greater than that of the corresponding period of a day of inactivity; and that some later period (almost always the one immediately following) exhibits with equal regularity an output unusually low. These opposing effects, which are not noticeably modified by fasting, compensate one another so closely that the net effect upon the output of 24 hours is negligible.

The temporarily increased creatinine excretion associated with work might be the consequence of an increased production of creatine within the contracting muscle, or of an accelerated transformation of the creatine already there, or of both these factors operating together. The choice between these possibilities would obviously be easier if we could learn by direct observation of the muscle itself what changes in its creatine content take place during contraction. Unfortunately no unanimity of opinion upon this point has yet been reached. It has been reported that stimulation of isolated frog muscle causes an increase of total creatinine (31); but also that it produces no effect (159), (175).

Stimulation of muscles in situ with the circulation of the blood intact has been said to bring about a decrease in the frog and in the rabbit (31); but the existence of any effect whatever under such conditions has been denied for the frog (175) and the cat (199). On the other hand cats' muscles stimulated in place after ligation of the main artery of the limb have been found to lose on the average 6.3 per cent of their creatine (199). If all the experimental data merit equal confidence, such divergence of result can have but one meaning—that in the experiments hitherto undertaken some controlling or modifying conditions have been overlooked. One cannot but be reminded in this connection of the perplexities that formerly beset the problem of the production of lactic acid in muscle; and it seems evident enough that the behaviour of muscle creatine during contraction will remain obscure, until it has been studied under conditions as precisely defined and as fully controlled as were those affecting lactic acid by Fletcher and Hopkins. It would be idle to speculate upon the facts as at present imperfectly known. One quite general deduction seems to be all that is justified; that the creatine of muscle is in *some* way, directly or indirectly, affected by muscular work. When the precise nature of the relation has been elucidated, we may be able to explain the temporary effects of work upon urinary creatinine.

THE FATE OF RETAINED CREATINE AND ITS BEARING ON CREATINE METABOLISM. We have seen that when creatine is introduced from without a certain amount is always retained in the body. The quantity which may be so retained is apparently rather limited, since even when presented with as much as 20 grams the human organism has not been observed to keep more than 5.7 grams (172). It is of interest to consider what may become of this.

Since it is certainly not destroyed, two possibilities only seem to present themselves. Either it may be deposited unchanged in the tissues, especially in the muscles, or it may be utilized in the synthesis of other substances. There is no doubt at all that the first of these alternatives can be realized. Polin and Denis (70) observed rapid increases in the muscle creatine of cats who were absorbing creatine from the intestine; in one of their experiments the creatine content of the muscle rose 26 per cent. Myers and Fine (143) were able in this way to account for from 22 to 100 per cent of the creatine retained by rabbits during continued subcutaneous administration; from which it would appear even that the *whole* of the retained creatine may sometimes be simply deposited in the muscles.

If creatinine is an index of muscle creatine, an increase of the latter brought about by the retention of ingested creatine ought to be reflected by a proportional increase in the output of creatinine, not only during the administration of the creatine, but for some time thereafter. Such a continued elimination of extra creatinine is not infrequently to be detected in the records of experiments. A striking instance is seen in the experiment of Rose and Dimmitt (172), as shown in table 2; on the fourth day after the last dose of creatine the creatinine was still decidedly above its normal level. The converse of this effect is possibly represented in the gradual fall of creatinine output which Ringer and Raiziss (166) observed to follow the complete withdrawal of creatine from the diet.

During the six days of creatine feeding in the experiment of Rose and Dimmitt the total amount of creatine retained was over 17 grams. Is it possible that this was simply added as such to the store of creatine already present in the body? As the subject weighed 54 kgm. his muscles probably contained 90 grams; 17 grams more would mean an increase of 19 per cent. The experiments of Folin and Denis, just quoted, show that as a temporary effect such an increase is possible; as a semi-permanent one it hardly seems likely. One is almost forced to assume that some of the creatine was utilized in synthetic processes. The same conclusion is indicated by the figures quoted from Myers and Fine, showing that even when the gain in muscle creatine is allowed for, as much as 78 per cent of the creatine retained may have apparently disappeared.

The evidence therefore suggests that the second alternative also is sometimes realized, that creatine may be of use in the anabolic processes of the body; may, as Folin (64) put it in 1906, serve as a food. In what manner it does so can only be guessed. Its chemical relationship to choline points to a possible use in the synthesis of lecithine; by its guanidine radicle it might be supposed to take part in the formation of arginine (158). If Cathcart (37) is right in holding that carbohydrate is necessary to its utilization, while fat is of no service, the latter supposition would be the more probable.

If creatine does function as an anabolite, its utilization is almost certainly accomplished entirely in the muscles. For in muscular atrophies or dystrophies 90 per cent or more of administered creatine, even of small doses, appears unchanged in the urine (122), (76).

**CREATINURIA.** While the urine of the adult male on a creatine-free diet contains no creatine, that substance is a normal and constant

associate of creatinine in the urine of children of either sex up to the age of puberty. In women also creatinuria is a physiological occurrence although only an intermittent one. Both in men and in women creatinuria may be induced or increased by a great variety of experimental or pathological conditions. The lower mammals behave in these respects very much like the human species, but exhibit still more readily both the spontaneous and the induced varieties of creatinuria. Ruminants (cattle, sheep and goats), for example, regularly excrete considerable quantities of creatine (153).

The most important forms of induced creatinuria are those produced by starvation, carbohydrate deprivation (including diabetes mellitus, pancreatic diabetes, and poisoning by phlorhizin, hydrazine (127), (205), adrenalin (204) or sodium selenite (41)), wasting diseases generally, exophthalmic goiter (182), (48), fever (109), (182), (183), muscular atrophies and dystrophies (122), (76) and perhaps acidosis. As it would be impossible to consider every one of these conditions, discussion will be limited to those bearing most directly upon the physiological problem.

*Creatinuria of starvation and carbohydrate deprivation.* The fact that complete fasting usually brings about an excretion of creatine was first observed almost simultaneously by Cathcart (37) and F. G. Benedict (22), (23) in man, and by Dorner (55) in the rabbit. Many others have since confirmed the observation for these species (98), (26), (135), (141) or extended it to others like the dog (205), (163), (97), (99), (100), sheep (101), pig (188) and guinea pig (155). In many of the reported observations, the disturbing effect of the concomitant ketonuria upon the analysis (78), (77), (29) has been unrecognized or disregarded, but the creatinuria is nevertheless a real one (40), (138), and the only uncertainty with which the data are sometimes affected is a quantitative one.

Different individuals and different species vary in the rapidity with which fasting creatinuria sets in, and in the intensity which it assumes. In the pig it is sometimes impossible to produce a starvation creatinuria at all (130), (188). Even the dog may at certain stages of a prolonged fast excrete no creatine (100). Such differences depend no doubt upon differences in the nutritive condition of the animal at the commencement of the fast (135), or upon its relative ability to utilize fat in shielding the tissues from disintegration (130).

The creatine output during starvation bears no constant relation to either the total nitrogen or the creatinine nitrogen. Sometimes there is observed a parallelism or even a strict proportionality between

total nitrogen and "total" creatinine (135), (141), and sometimes the latter maintains an almost constant value throughout the fast, as if to suggest that fasting merely diminishes the proportion of waste creatine transformed into creatinine (22); but neither of these phenomena is a regular occurrence (218).

Starvation involves the consumption by the organism of its own protein, probably in the first place chiefly the "reserve" or "circulating" protein, but as this diminishes an increasing proportion also of the "tissue" or fully organized protein. The latter must be furnished largely by the muscles, which lose 42 per cent of their weight in a dog starved for twenty-four days. As the muscle disintegrates, its creatine, it is reasonable to suppose, will be liberated. Such internally liberated creatine might be expected to behave just like creatine introduced from without; in which case, since the starving organism is living on a fairly high protein level, some of it would be retained, but some excreted unaltered. The retained creatine would in part perhaps be utilized, in part simply deposited in the remaining muscular mass, the creatine content of which is actually found to be raised during the earlier part of a fast (136), (141). In the later stages of starvation creatine for some reason, totally unexplained as yet, is lost even from that muscle tissue which remains apparently intact (97), (141); this might explain why the creatine output rises disproportionately towards the end of the fast (100).

The direct derivation of urinary creatine from the preformed creatine of muscle was suggested by F. G. Benedict (22), was admitted by Mendel and Rose (135) as a contributing factor in hunger creatinuria, and was definitely adopted by Myers and Fine (141) as a complete explanation of that phenomenon. This view has an obvious simplicity in its favour which would make one loath to abandon it until it has been shown to be incompatible with the facts. It is supported by such an observation as that of Frontali (73) who found that the marked creatinuria which follows total thyroidectomy in dogs is accompanied by (and probably due to) a very considerable loss of creatine from the muscles. It is not invalidated by the fact that the amount of muscle disintegrated, as calculated from the output of creatine, does not often agree with the amount calculated from the total nitrogen loss. As a matter of fact muscle loss cannot be calculated from either of these data. The nitrogen of the urine is in part derived from sources, such as reserve protein or organ protein like that of liver, which yield little or no creatine, a factor which would make the proportion of creatine to total nitrogen lower in

urine than in muscle, as is usually the case. On the other hand the protein nitrogen of the disintegrated muscle may be only in part excreted, in part utilized in more essential tissues by synthetic processes which do not involve the simultaneously liberated creatine; in such circumstances the creatine output would tend to outstrip the total nitrogen, as it sometimes actually does (99). Paton (156) in 1910 not only pointed out these theoretical possibilities but attempted to illustrate them by experiments upon ducks fasted after different types of feeding and starting therefore with different amounts of surplus protein. Still other factors disturbing the relation between total nitrogen and creatine are the possible retention of muscle creatine, suggested above, and the selective loss of creatine from muscle tissue which seems to take place in the terminal stages of a fast.

Before weighing certain objections that have nevertheless been taken to Myers' and Fine's explanation of starvation creatinuria, it is necessary to consider some other aspects of the phenomenon.

In 1909 Cathcart (38) showed that the creatinuria of starvation was promptly abolished in man by the administration of a practically protein-free carbohydrate diet, but not (as he thought) by the administration of fat with protein. The effect of carbohydrate was confirmed by Mendel and Rose (135) for rabbits, and by Wolf and Osterberg (218) for dogs. Cathcart offered, to explain his results, the hypothesis that creatine is produced in the organism in considerable amounts, but is normally utilized in synthetic processes for the accomplishment of which carbohydrate is indispensable; in the absence of sufficient carbohydrate, as in starvation, the creatine, which cannot now be utilized, is excreted. Mendel and Rose, who like Cathcart found protein and fat to be ineffective, agreed that carbohydrate is specifically essential to normal creatine metabolism, but suggested that it might be concerned in the transformation of creatine into creatinine. It presently appeared, though, that the effect of carbohydrate in preventing inanition creatinuria was not so specific as had been thought. Graham and Poulton (77) showed that mere deprivation of carbohydrate, as brought about for a few days at least by a diet of fat or fat with protein, does not lead to an excretion of creatine in man; the creatine observed by Cathcart upon such diets being shown to be probably an analytical error due to ketonuria. Wolf and Osterberg (218) had already shown that in dogs serum protein will abolish the creatine of starvation almost as effectively as starch; and Rose, Dimmitt and Cheatham (171) proved that a diet of eggs (protein with fat) will do the same for man. In the

pig a pure fat diet, even if continued for a long period, does not necessarily lead to creatinuria (131), nor does a pure starch diet necessarily prevent it (188). The effect of carbohydrate therefore is neither unique nor certain. It is possible that it is, as Myers and Fine suggest (142), merely one phase of the well known sparing action of carbohydrate upon protein catabolism. The apparent impossibility of abolishing starvation creatinuria by fat alone (218) may be due partly to its relative inefficacy as a sparer of protein, partly to the acidosis which it induces, partly to the practical difficulty of feeding enough.

There are, nevertheless, certain other facts which are believed to speak for a special connection between creatine metabolism and carbohydrate. If the body tissues are deprived of carbohydrate by phlorhizin (39), (114), (115), (217), (135), (28), or lose the ability to utilize carbohydrate as in diabetes mellitus (14), (115), (191) or pancreatic diabetes (170), creatine is invariably excreted. The amounts found appear to bear an intimate relation to the extent to which carbohydrate is actually withdrawn from the tissues; in phlorhizinized starving dogs they become relatively enormous. Of course the conditions named involve not merely withdrawal of carbohydrate, but also as a rule a concomitantly increased catabolism of protein, and, as far as the data permit one to judge, the creatinuria seems under most conditions to run parallel with the latter (39), (217). S. R. Benedict and Osterberg (28) claim to have shown that it is none the less entirely independent of tissue destruction; and, as their observations constitute by far the most formidable difficulty in the way of identifying the urinary creatine of starvation or carbohydrate deficiency with preformed creatine set free from disintegrated or altered muscular tissue, they must be carefully considered.

Benedict and Osterberg found that it was possible to feed to fasting phlorhizinized dogs such amounts of creatine-free protein as would nearly or altogether abolish the negative nitrogen balance. They argue thereupon that "if the creatine of the urine has its origin in the destruction of muscular tissue . . . , a sparing of the body tissue destroyed by feeding exogenous protein should cause a corresponding fall in the creatine eliminated, whereas if the utilization (or destruction) of creatine be dependent upon carbohydrate utilization, the ingestion of exogenous protein in the phlorhizinized dog should not appreciably affect the output of creatine in the urine." The experimental results are held to settle quite definitely the choice between these alternatives. The creatine output, it is claimed, reveals itself as totally independent

of the state of the nitrogen balance. "Creatine may be eliminated in the urine in large amounts without any corresponding loss of body tissue." The creatine excreted cannot therefore simply represent preformed muscle creatine liberated by the dissolution of flesh. Creatine, it is concluded, must be constantly "formed in the animal organism in relatively large amounts," but "is normally for the most part either utilized or destroyed." This disposition of the creatine is dependent upon the utilization of carbohydrate, and when that is impossible, as in phlorhizinized animals, the constant production of creatine is revealed by its elimination. The success of protein in preventing creatinuria in normal dogs is attributed to its capacity for the formation of glucose; its failure in phlorhizin poisoning to the withdrawal of even proteinogenous glucose from the tissues.

It is doubtful if Benedict and Osterberg's data furnish adequate proof of these propositions. They demonstrate at the utmost nothing more than the production, independently of tissue catabolism, of notable amounts of creatine in *phlorhizinized animals*. To infer a similar extensive production in normal animals is hardly justifiable. The production in phlorhizin poisoning of excessive amounts of sugar from protein does not prove that the metabolism of amino acids in the normal organism necessarily involves the intermediate production of glucose. To show, by a drastic interference with normal metabolism, that the dog possesses the capacity to form more creatine than it can utilize, does not prove that under physiological conditions it exerts that capacity any further than the need of the organism for creatine may require. The results of Benedict and Osterberg are striking and important, and must be seriously reckoned with; but to the writer they seem to leave still open the questions of the relation between starvation creatinuria and muscle waste, and of the unique importance of carbohydrate in creatine metabolism. Their confirmation, upon pancreatectomized dogs, by Rose (170) adds of course nothing to their real significance.

It may be added that Cathcart, if the writer understands him correctly, has receded somewhat from his earlier views, and now regards the output of creatine in conditions of carbohydrate deficiency not so much as evidence of the disturbance of creatine metabolism *per se*, but rather "as an index of faulty metabolism in general" (41), (42); and he seems inclined to relate it, sometimes at least, directly to the creatine content of the muscle tissue catabolized (41).

*The creatinuria of high protein feeding.* In 1912 Folin and Denis (65) suggested as an explanation of the creatinuria of children that it is due to an excessively high level of protein consumption in proportion to muscular mass; but McCollum and Steenbock (130) appear to have been the first to definitely postulate an *exogenous* as well as an endogenous origin of urinary creatine, and to produce experimental evidence in its support. They came to the conclusion that not simply the plane of protein intake, but the character of the proteins in the diet, determines the extent of creatine production. They found, for example, that an abundantly fed pig receiving five times its endogenous protein requirement in the form of linseed and gluten meals excreted regularly considerable quantities of creatine, whereas if it received the same quantity of protein or even twice as much from corn alone the urine seldom contained any creatine at all. Steenbock and Gross (188) have more recently shown that in fasting pigs the administration of casein in sufficient amount will induce creatinuria when that is absent, or increase it when already present. This is in remarkable contrast to the observed inhibitory effect of serum or egg proteins upon starvation creatinuria in dogs (218) or men (171), and emphasizes the probable importance of the kind of protein fed.

Further evidence for an exogenous origin of urinary creatine has been found by Denis and her associates in the facts, *a*, that the output in a variety of conditions (exophthalmic goiter, childhood, etc.) appears to bear a relation to the meals (always creatine-free) of the subject, being very much smaller at night than during the day, and usually attaining a maximum in about two hours after the substantial meal of the day (47), (49); *b*, that the amount of creatine excreted by cases of exophthalmic goiter (male or female) is increased by high protein feeding and decreased or reduced to zero by a low protein diet (48); *c*, that the creatine output of children is increased by a diet rich in protein, and diminishes or even disappears upon a minimum protein intake (49); *d*, that creatinuria can be produced in adult women by forced protein feeding and made to disappear again by lowering the protein intake (50), (52). Gibson and Martin (76), again, found that the creatine output in pseudohypertrophic muscular dystrophy is intimately related to the protein intake, their data being of particular interest because in the condition named ingested creatine is excreted quantitatively, so that their subject was practically "diabetic" as regards creatine. They made the further very important observation that it is only the exogenous protein immediately catabolized, and not that retained for growth that affects the creatine output.

The view that creatine may, in part at least, have an exogenous origin was supported by Harding and Young (85) on the basis of data (as yet unpublished) obtained from growing puppies. On the other hand Powis and Raper (162), who first observed the periodic variations in the creatine output of children, found these quite unrelated to meals; Rose, Dimmitt and Bartlett (173) were unable to induce creatinuria in women by high protein feeding; and in the case of normal men it has not been found possible to bring about on the highest attainable plane of protein intake (even with 33 to 35 grams of nitrogen in the urine) any excretion of creatine whatever (50). Such failures do not destroy the significance of positive observations; but they emphasize the difficulty of producing creatinuria by protein feeding alone.

It is of course an apparent inconsistency that protein may sometimes, as when fed to starving dogs or men, abolish an existing creatinuria, and sometimes, as in the experiments of Denis and her collaborators, induce one where it was absent. If it is true that only that protein is effective which is catabolized, the inconsistency becomes less glaring; for much of the protein fed after a period of starvation may be simply retained in the body, as was certainly the case with the dogs of Wolf and Osterberg (218). This conception may also explain why differently constituted proteins should vary in their effect. The data for the experiments of Denis and Minot upon women (50) do not permit us to calculate exactly the nitrogen balance, but as their diets contained a large amount (50 grams) of gelatin, it is probable that the proportion of food nitrogen promptly excreted by their cases was relatively high. This may account for the difference between their results and those of Rose, Dimmitt and Bartlett.

Although an effect of food protein upon creatine production appears to have been fully demonstrated, this is not necessarily to be interpreted as proving an exogenous source for creatine, in the sense that the latter may arise directly, like urea, from certain precursors in the ingested protein molecule. There may be another explanation of the phenomenon. It may represent merely one phase of that general stimulation of cellular metabolism which is described as the specific dynamic action of protein. An increased *endogenous* production of creatine, resulting from such stimulation, might be expected to manifest itself in just the sort of creatinuria that follows protein ingestion. It would be brought about only by catabolized protein, since protein deposited in the form of new tissue exerts no specific dynamic action (125); it would be at its height, as Denis and Kramer found it to be (49), during the second and

third hours after ingestion, when heat production has reached its maximum but the output of urea is still rising (212); and it would occur most readily in those conditions where the metabolism is already relatively high, as in exophthalmic goiter, or in childhood. Such an explanation of the creatinuria following a high protein diet seems therefore to be well worthy of consideration. Protein feeding will increase also the output of uric acid; but no one has sought the origin of uric acid directly in the protein of the diet. Lewis, Dunn and Doisy (123) have shown reason to believe that proteins and amino-acids increase the production of endogenous uric acid by virtue of their general property of stimulating all cellular metabolism; it does not seem improbable that they should simultaneously increase the production of endogenous creatine.

*Creatinuria in children.* The fact that normal infants and children of both sexes usually excrete creatine as well as creatinine was discovered by Rose (168), confirmed by Folin and Denis (65) and by Krause (118), and has formed the subject of further study by Powis and Raper (162), Denis and Kramer (49) and Gamble and Goldschmidt (74). The creatinuria in question is of course a physiological phenomenon, and must depend upon quantitative rather than upon profound qualitative differences between child and adult. Probably it represents a temporary survival of an earlier stage in the evolution of the type of creatine metabolism which now characterizes adult man.

The precise point, or points, in the metabolic cycle, at which children differ from adult men, is not quite clear. It might be that children produce relatively more creatine; or that they produce, relatively to their muscle mass, the same quantity of creatine as adults, but have a less completely developed capacity to retain (or assimilate) it. Actually the creatine content of the immature muscle is lower than that of the fully developed (see table 1), as if it were more readily "saturated" with creatine; and in accordance with this conception children are found to excrete a relatively large proportion of ingested creatine (118), (162)—infants as much as 100 per cent (74)—just as do the subjects of muscular dystrophy (122), (76). The avidity (if it may be so expressed) of the muscle for creatine and its efficiency as a machine develop together, and as they develop creatine disappears from the urine. It is also possible that children have a relatively low power to convert muscle creatine into creatinine. On this point we have as yet no very direct evidence.

*Creatinuria in women.* In contrast to normal men, normal women even upon a creatine-free diet excrete, although only intermittently, small quantities of creatine. This was first observed by Krause and Cramer (116), (117), who thought that the creatinuria had a definite relation to the menstrual period. Others (167), (173), (187) have been unable to observe any regularity whatever in the output.

It appears not unreasonable to suppose that the creatinuria of women represents merely an imperfect transition from the creatine metabolism of childhood to that of vigorous adult life, and that it is associated with the relatively poor muscular development and low creatinine coefficient of the female sex in general. This would imply that the female organism has a lower power of assimilating creatine than the male. The experiments of Stearns and Lewis (187) upon the fate of ingested creatine in women seem to show that this is not the case. It is to be noted, though, that the two subjects they studied did not excrete spontaneously more than a trace of creatine, and that indeed their urine was often creatine-free for weeks together. In other words these women, in regard to creatine metabolism, gave hardly any indication that they differed from men. Perhaps, if the point were examined, it would be found, as Stearns and Lewis seem to infer, that women leading a life of abundant muscular activity exhibit no creatinuria at all.

The generally intermittent creatinuria of women becomes continuous during pregnancy (114), (117), (95), increasing toward the end so that in the last few weeks before parturition it may average 0.17 gram daily (211). This may be correlated in some way with the fact that the uterus during pregnancy acquires both absolutely and relatively more creatine than the resting organ (21). Immediately after delivery the output of creatine rises further still, sometimes reaching the extraordinary figure of 1.5 grams in 24 hours (182), and averaging 0.42 gram daily in the first four days of the puerperium (211). According to Heynemann (90), this post-partum creatinuria is at its height, in the human subject, on the third and seventh days; in the dog Murlin (139) found a maximum on the fifth day. Shaffer (182) and Murlin (139) attributed the phenomenon to the rapid escape of creatine from the involuting uterus. This explanation is extremely plausible, and one can hardly doubt that it correctly states one factor at least; but it does not seem to be certain that the gravid uterus contains enough creatine to account for the total quantity excreted. Beker (21) calculates that the human uterus in returning to the non-pregnant condition loses 0.73 gram of creatine; the total excess output of the puerperium must

often be greater than this. Mellanby (134) brings the post-partum creatinuria rather into relation with the onset of lactation. The excretion of creatine during pregnancy and the puerperium is in obvious need of further and more detailed study.

*Creatinuria and acidosis.* A great many of the conditions resulting in creatinuria—starvation, carbohydrate deficiency, diabetes, fever, etc.—are characterized by an abnormal production of acetoacetic acid or other acid substances, and therefore by a tendency to depletion of the alkaline reserve, or acidosis. This suggested to Underhill (206) in 1916 the hypothesis “that a condition of acidosis in the body is responsible for the appearance of creatine in the urine.” After testing this hypothesis in a variety of ways upon rabbits (207), (208), (209), he concluded that there is indeed an interrelationship between acidosis and creatine elimination, but that neither acidosis nor carbohydrate deficiency can be the sole factor in the production of every type of creatinuria. A similar conclusion is reached by Steenbock and Gross (188) on the basis of experiments upon pigs. Denis and Minot (52), on the other hand, could observe no definite effect upon creatine output on administering sodium bicarbonate to boys or women. Sawyer, Stevens and Baumann (174) produced in children a demonstrated reduction of alkaline reserve by giving a high-fat low-carbohydrate diet; the creatine output was always increased, but the authors themselves do not believe that the acidosis in itself was the responsible factor. Gamble and Goldschmidt (74) were also unable to secure, by adding acid or base to the food of infants, any evidence of a relation between creatinuria and the acid-base balance of the diet.

It is quite certain that acidosis is not in any condition at present known the sole determining cause of creatinuria, and it is doubtful if it is often an important contributing one. That it may sometimes play a subsidiary part is not impossible, and may even, in view of its stimulating effect upon cellular metabolism, be considered likely.

Upon a general review of the whole situation with regard to creatinuria it appears very improbable that the condition can be referred in all of its types to any single cause. Probably creatinuria is always endogenous in origin and most examples, if not all, could be included under one or more of the following descriptions:

1. Creatinuria due to dissolution of muscular tissue; as in starvation, carbohydrate deficiency, etc.

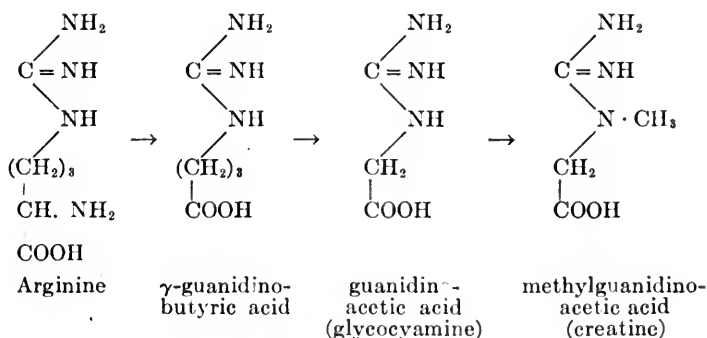
2. Creatinuria due to over-stimulation of endogenous metabolism; as in fevers, exophthalmic goiter, high-protein feeding, etc.

3. Creatinuria due to defective power to store, or utilize, or dehydrate creatine produced in normal amounts; as in childhood, muscular dystrophies, etc.

ORIGIN OF CREATINE. It is generally assumed, and the assumption has every element of probability in its favor, that creatine is derived from protein. Of the mechanism of its origin and of the nature of its immediate precursors we possess no certain knowledge. None of the supposed precursors which have hitherto formed material for experiment has been shown to satisfy the two requirements necessary to establish it in the status of an actual intermediate: *a*, that it should, upon administration in the proper way (that is, in such a way as to reach the actual locus of transformation), be converted readily and abundantly into creatine (or creatinine); and *b*, that it should be detectable in traces at least as a normal constituent of the body or of the urine. If creatine were a waste product the almost uniform failure to connect it with hypothetical precursors would almost prove that the real precursor has not yet been thought of. As we have seen, creatine is probably not a waste product, but either an integral part of the living protoplasm (70), or a tissue constituent with a special function. Its rate of production is therefore in all likelihood regulated by the internal demand, and it is not to be expected that it should be accelerated by an excessive supply of precursors, any more than the production of adrenalin or thyroxin would be increased by the administration of large doses of tryptophane. One encounters rather frequently, it is true, the idea that creatine is constantly being produced in relatively large quantities, and as constantly in some mysterious way being "destroyed." The experimental work reviewed in the preceding pages affords no proof of, and little support for, such a conception. We have positive knowledge of creatine production only in connection with the growth or renewal of protoplasm in certain tissues; and creatine is not "destroyed" in any other way than by conversion into creatinine, although it may possibly be utilized in unknown anabolic phases of metabolism. Folin and Denis (70) explain the failure to trace creatine to any known food constituent by an essentially identical argument.

Speculation and experiment upon the origin of creatine have centered mainly upon arginine, the only known protein constituent possessing a guanidine radicle.

1. *Arginine as the mother substance of creatine.* On the theory of Knoop (110) and Neubauer (149) creatine arises from arginine by the following series of reactions:



Up to the formation of glycoeyamine this scheme follows the well established lines of amino-acid catabolism by deamination and  $\beta$ -oxidation. The final step in the process—the methylation of glycoeyamine—is one which, it is generally agreed, the animal body has been demonstrated to be capable of taking (106), (55), (154), (197), (16), (76). The scheme has therefore a certain plausibility, increased rather than diminished by the fact that, as Neubauer himself points out, it could not be expected to apply to *ingested* arginine, the guanidine group of which would be converted into urea by the arginase of the liver, but would readily account for the appearance of creatine in the special metabolism of muscle, where arginase is not to be detected.

Attempts to secure direct evidence of the conversion of arginine into creatine have for the most part given admittedly negative or inconclusive results (106), (13), (17), (195). Positive results are reported by Inouyé (104), who obtained small increases of total creatinine upon incubation of arginine with hashed liver, or perfusion through the surviving organ; by Thompson (197) who observed increases of urinary creatine in dogs, birds and rabbits, after injection or oral administration of arginine; and by Gross and Steenbock (81) who confirmed Thompson's results by feeding the base to pigs. The significance of Inouyé's data is doubtful; but as the arginine in his experiments must have been promptly hydrolyzed by the liver arginase, its guanidine group can hardly have been responsible for any observed formation of creatine. In Thompson's experiments the extra creatine never corresponds to more than a small fraction—from 1.1 to 4.5 per cent—of the guanidine nucleus introduced; no control experiments were made with other amino-acids; and it is not at all impossible that what was observed was a general amino-acid effect of stimulation. The data of Gross and Steenbock distinctly support such an explanation; for while they

observed increases of urinary creatine upon feeding arginine, they obtained increases greater still from casein of equivalent arginine content. None of these experiments therefore furnish conclusive evidence of a conversion of arginine into creatine. Evidence more direct is possibly to be found in experiments of Jansen (108), who reports that increased tonus of frog's leg muscle is accompanied by a disappearance of arginine and the production of a corresponding amount of creatine.

As might be expected no unequivocal effect upon muscle creatine (146), or upon creatine excretion (86), (76), or creatinine output (93) is produced by exchanging an arginine-poor protein in the diet for an arginine-rich one.

In the endeavor to establish a connection between creatine and arginine, experiments have been made also with many possible intermediates and their higher homologues. Neither  $\gamma$ -guanido-butyric (192) nor  $\epsilon$ -guanidino-caproic (193) acids, nor the corresponding methylamino or methylguanidine derivatives (194), nor yet  $\delta$ -methyl-arginine (195) give any evidence of convertibility into creatine in the animal organism. Among all the conceivable intermediates of an arginine-creatine transformation glycoeyanine remains the only one which gives positive results; and there are difficulties in accepting it as a real intermediate of normal metabolism. It is for the most part excreted unchanged, only a fraction of the administered dose undergoing methylation; and although only slightly soluble, it has never been detected as a constituent of animal tissues.

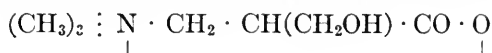
In the face of such almost uniformly negative results one is almost forced to assume that, if creatine is related to arginine at all, its mother substance is not free arginine but the still combined arginine of the muscle protein. Seemann's (180) hypothesis of the existence in protein of a preformed creatinine ring, in the construction of which the guanidine group of arginine is supposed to participate, contains an inherent improbability; for all the evidence indicates that the guanidine groups of the protein molecule are free (113). It is more likely that, as Thomas (195) suggests, the projecting arginine side-chains undergo in tissue metabolism  $\beta$ -oxidation from the terminal guanidine group inwards, the disrupted fragment being then converted into creatine. Such a speculation readily falls in line with the view of McCollum (129) that endogenous metabolism does not involve the complete disintegration of the protein molecule. Complete disintegration of the molecule would indeed be difficult to reconcile with an origin of creatine (and creatinine) from arginine alone; for arginine contributes but 11.1 per

cent, its guanidine nucleus therefore 8.3 per cent, of the nitrogen of muscle and tissues in general (56), whereas, when the metabolism of protein is reduced to a purely endogenous level, creatinine may form 18.5 per cent of the total nitrogen output (128).

Support for the conception that creatine is derived from arginine has been found in the fact that while the muscles of vertebrates contain creatine but no free arginine, those of invertebrates (crustaceans, insects, molluscs), which contain no creatine, yield larger or smaller quantities of free arginine (2), (4), (6), (7), (119), (152).

2. *Creatine as a detoxicator of guanidine.* Noel Paton (158) has recently revived the hypothesis of Jaffé (106) and Achelis (1) that creatine is the product of a reaction having for its object the detoxication of guanidine or methyl-guanidine. Guanidine is presumed to arise from the complete oxidative decomposition of arginine, from some other unknown guanidine grouping in the protein molecule, or even under certain circumstances—as in the developing chick (34)—from non-guanidine nitrogen. Methyl-guanidine is generally believed to be a normal constituent of flesh (185) and urine (57). Both bases are highly toxic, producing symptoms similar to those of tetany (157). In tetaniaparthyreopriva (112), (33) and in idiopathic tetany (33) their concentration in blood or urine or both is greatly increased. After removal of the parathyroids the creatine content of muscle increases while its total guanidine concentration falls (87). In spite of negative results (chiefly with methylguanidine) by others (106), (55), (164), (1), (17), Thompson (198) found an increase of creatinine output in the dog, and of creatine output in the duck, while Wishart (215) obtained increases of muscle creatine, after injections of guanidine salts. When it is considered that it is by no means certain (59), (18), (80) that the methylguanidine found in flesh and elsewhere is not an artificial product, the evidence hardly seems to form a very strong case for the detoxication theory.

3. *Creatine as a product of methylation.* The derivation of creatine from arginine or from the guanidine nucleus of protein involves at some point or other a process of methylation. Methylation and methylated compounds are specially characteristic of the vegetable kingdom (witness the numerous plant betaines and methylated alkaloids), but are by no means uncommon among animals. Choline (as a constituent of lecithine), adrenalin, carnitine (probably

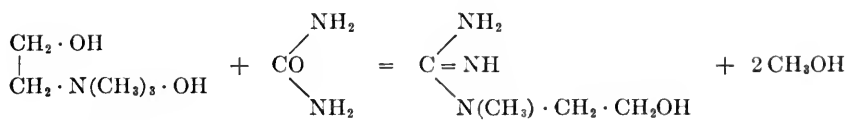


(58) and glycocoll-betaine are examples of methyl compounds with wide distributions in the animal kingdom.  $\gamma$ -Butyro-betaine is found in the urine of dogs poisoned by phosphorus (190). Pyridine (91), (96), (201), tellurium salts (92) and nicotinic acid (3) given by mouth are excreted as methyl derivatives. The instance of glycocyamine and its conversion to creatine has already been noted.

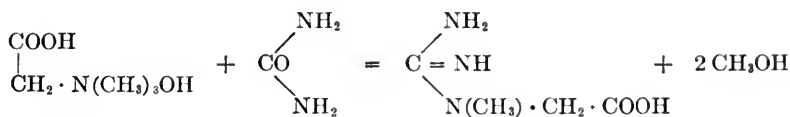
Neither the purpose nor the mechanism of methylation are perfectly clear. It has been thought that it serves to protect the substance involved from further catabolism. Certainly the betaines and methyl compounds in general are very resistant to oxidation in the animal organism (5), creatine, as we have seen, being no exception to this rule. It is possible then that the formation of creatine serves to preserve for special uses the guanidine group of arginine.

In plants methylation is believed to be effected by the union of formaldehyde with amino groups, and reduction of the methylene-amino compounds thus produced. Thompson (200) has suggested that creatine may arise in a similar way by the action of formaldehyde upon guanidino-acetic acid or some earlier stage in the oxidative catabolism of arginine. He supported his hypothesis by experiments in which the administration, especially the subcutaneous administration, of paraformaldehyde or urotropin with or without arginine to ducks appeared to give notable increases in the output of creatine. It may be objected that formaldehyde is not known to arise in the animal body, that if it did there seems to be no reason why it should couple with guanidine rather than with the far more abundant free amino groups in the tissues, and that Thompson took no account in his analyses of the fact that formaldehyde gives the Jaffé reaction. There seems therefore to be no real evidence that creatine formation represents a mechanism for the detoxication of formaldehyde, or that the latter has anything to do with its origin.

4. *Creatine as a derivative of choline or betaine.* Koch (111), in 1905, suggested that creatine as a methylated amino-acid derivative might be related to the metabolism of lecithine, of which the methylated amino-alcohol choline is a characteristic component. Riesser (164) in 1913 developed this hypothesis in considerable detail, pointing out that not choline alone but also its oxidation product betaine might be supposed to be capable of condensing with urea, according to the following schemes:



Choline



Betaine

Creatine

Betaine would thus give creatine directly, while the condensation product with choline would yield it upon simple oxidation. On this theory the guanidine group of creatine would be formed synthetically, and not derived from any preformed residue in the protein molecule.

In support of his hypothesis Riesser reports experiments upon rabbits indicating that both the creatine content of muscle (164), and the daily output of creatinine (165) may be increased by subcutaneous injections of choline, or of betaine. Thompson (198), on the other hand, found no very decided effect of either substance upon total urinary creatinine in the dog; and Baumann and Hines (17), who perfused urea with betaine or choline through dog's muscle, obtained no increase of creatine with the former and only a doubtful one with the latter. The experiments of Riesser therefore, although exceedingly suggestive, do not suffice to establish either choline or betaine as precursors of creatine.

It might be pointed out that betaine at least, in the rôle of a creatine precursor, would not have any necessary connection with lipid metabolism, but as a fully methylated glycocoll might equally well be derived from protein. Indeed it is almost certain (11) that the betaine found in living organisms arises not from choline but from glycocoll. As glycocoll can apparently be produced in almost unlimited quantities in the endogenous metabolism of protein (at the expense of a corresponding quantity of urea) (132), it is unnecessary to look elsewhere for a constant source of betaine in Riesser's hypothetical reaction. Now it is of no little interest that while betaine is absent as a rule from the creatine-containing muscles of vertebrates, it has been rather frequently found in those of invertebrates. It has been detected in the mussel (30) (7), the shrimp (2), the crayfish (119), the octopus (88), the scallop and the periwinkle (213), the spiny lobster and *Loligo* (152), none of which yield even traces of creatine. It would seem to be of

particular importance that in some of the lowest vertebrates, for example the lamprey (213) and the dogfish (*Acanthias vulgaris*) (189), creatine and betaine are found together. Such data of comparative biochemistry, scattered and incomplete as they are, certainly suggest that betaine is a step in the evolution, and probably therefore in higher forms in the production, of creatine. The occurrence of betaine in ox-kidney (19), if it should be confirmed, makes its position as a real intermediate still more probable.

Betaine as an intermediate product is of course not incompatible with arginine as the original mother substance of creatine, for arginine accompanies betaine in many invertebrates, and betaine might conceivably take origin from ornithine, through the preliminary stages of  $\gamma$ -amino-butyric acid and glycocoll. Methylation might be the final step, or might occur at either of the earlier stages. Carnitine, a regular constituent of vertebrate muscle, was at one time thought to be the hydroxy-derivative of methylated  $\gamma$ -amino-butyric acid, and as such might have figured as a possible relation of creatine; but it appears to have in reality a different constitution (58).

5. *Creatine as a derivative of cystine.* Harding and Young (86) have suggested that creatine may be derived from cystine "through the intermediate stages of taurine and amino-ethyl alcohol, followed by methylation, combination with urea, and oxidation." They have not yet published the experiments which led them to this hypothesis. Gross and Steenbock (81), who observed increases of creatine in pigs after feeding cystine, attributed their results to acidosis consequent upon the oxidation of the sulphur. Gibson and Martin (76) found cystine to be without effect upon the creatine output in pseudohypertrophic muscular dystrophy. It seems unlikely, in any case, that cystine could be the source of all the creatine produced in the body.

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# PHYSIOLOGICAL REVIEWS

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No. 1

## THE SECRETION OF GASTRIC JUICE IN HEALTH AND DISEASE

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I. METHODS: 1. *Methods of securing the gastric secretion.* The advances in our knowledge of gastric secretion run parallel with the development of new experimental methods and the degree of intelligence with which these methods are applied, experimentally and clinically. Reaumur and Spallanzani introduced food and sponges in perforated metal or wooden capsules into the stomach of man, birds and fishes. These capsules were recovered by means of attached strings, by vomiting, or by passage per rectum. By diligent application of these simple methods Reaumur and Spallanzani demonstrated *a*, that gastric digestion is a process of chemical solution rather than mechanical trituration; *b*, that gastric juice is acid; *c*, that it prevents putrefaction; and *d*, that it digests food in vitro.

The next step in advance was a chemical one, taken by Prout and Tiedemann and Gmelin. They secured gastric juice or gastric content by killing their animals after introducing pebbles and other indigestible material into the stomach, or at the height of digestion of a meal, and proved by adequate methods that the acidity of the gastric juice is due to free hydrochloric acid.

Next came data from clinical and experimental gastrostomy. While Beaumont was not the first to note gastric digestion and secretion in human gastric fistula cases, he was the first to make serious use of such a case to advance our knowledge of gastric physiology and pathology. Moreover, his classical research on Alexis St. Martin became the most important impetus to the work on gastric physiology in the 19th century. Beaumont secured pure gastric juice, but the limited chemical examination of this juice was made by others. Beaumont concluded, however, that gastric juice contains other food solvents besides the HCl. The substance hinted at was pepsin, discovered by Wassman six years

later (1839). Beaumont recorded that irritating condiments, excess of alcohol, febrile states, anger and fear, etc., decrease gastric secretion, facts that have been confirmed and extended by later workers. But Beaumont is also partly responsible for promulgating and perpetuating two erroneous views concerning gastric secretion, viz., that there is no secretion of gastric juice in the absence of food in the stomach, and that foods or indigestible solids induce secretion by their mechanical action on the gastric mucosa. But the blame for the perpetuation of these errors rests heavier on the followers than on the master, Beaumont. Since the publication of Beaumont's great work nearly ninety years ago important contributions have been made to gastric secretion on many other human gastrostomy cases, both in health and disease.

Simple gastrostomies on experimental animals quickly followed the publication of Beaumont's observations in 1833 (Bassow, Blondlet, 1842), but these led to no discovery of fundamental importance except the demonstration of Bidder and Schmidt that the HCl is actually produced by the gastric glands. The next important step, again a new method, was taken by Heidenhain in making the isolated pouch, or little stomach separated from the main stomach, so that pure gastric juice was for the first time obtained in the experimental animal parallel with normal digestion. Heidenhain's pupil, Pavlov, in the early nineties, made the important addition to the Heidenhain operation of so cutting the gastric walls that most of the extrinsic and intrinsic nerve connections were retained in the isolated gastric pouch. In this way Pavlov and his pupils were able to begin the analysis of the chemical and the nervous factors of the gastric secretion. The later development of Pavlov's research in the direction of conditioned reflexes deal with the central nervous system rather than with gastric functions.

*The stomach tube,—Fractional analysis.* The discovery and application of the principle of the stomach tube or stomach pump appears to go back into antiquity (Garrison). In modern medicine the stomach tube was first used for tube feeding and for gastric lavage (Monroe, Physick, Hunter, Kussmaul). But shortly after Kussmaul's first publication on the subject in 1867, Leube and Külz made use of the stomach tube for the purpose of securing gastric content and analysis of gastric secretion. As quoted by Maly, these investigators introduced the principle of fractional analysis, that is, removal of small portions of the test meal at fixed intervals, in order to follow the secretion process by the variations in acidity and pepsin content. This method was also followed by Schüle, who in addition showed that the appetite secretion of gastric

juice may be studied in intact persons by emptying the stomach, chewing palatable food without swallowing for fixed periods, and withdrawing the juice secreted. Following the lead of the great German gastroenterologists, most of the clinical work on gastric secretion during the last fifty years has been done by the less satisfactory method of withdrawing the entire gastric content at a fixed interval (usually one hour) after ingestion of the test meal. This procedure affords no control on the continuous secretion before ingestion of the food, and no adequate information on the secretion rate following the evacuation of the meal. The great body of data in the clinical literature on gastric juice secured by this method is therefore of doubtful value, even for diagnostic purposes. To be sure, Ewald, Boas, Reichmann and others attempted fractional studies by giving the same test meal on succeeding days or by removing the entire gastric content at different intervals after ingestion.

The large caliber and relatively inflexible catheter usually employed as stomach tube is too uncomfortable to retain in place for any considerable period, and too difficult to swallow so that passing this tube into the stomach at 15- or 20-minute intervals is out of the question. The motor and secretory disturbances induced reflexly by this procedure would vitiate the results. Delicate flexible catheters that could be swallowed with ease and retained in place with comfort over long periods have been used from time to time and were recommended for the study of gastric content by clinicians both in this country and in Europe. Thus in 1893 Gross reported the use of a soft nelaton catheter for gastric analysis. But these methods received little attention until about a decade ago when the modern method of fractional gastric analysis was introduced by Ehrmann, Ehrenrich and Ettinger, in Germany, and a little later by Rehfuess and his co-workers in America.

The adaptation of the duodenal catheter of Einhorn for the purposes of recovery of the gastric content, continuously or at short intervals, is a noted advance as regards the study of gastric secretion in man both in health and disease. The main shortcoming of the fractional method in connection with the test meal is due to the variation in degree of acidity of the gastric content in different regions of the stomach (Gorham, Kopeloff, White, Fitz).

Taylor has described an ingenious device for securing pure gastric juice on "sham" feeding in normal infants. The stomach tube is passed through a rubber tube, the latter ending in the esophagus. This outer tube is connected with a suction apparatus so that the milk or other liquids swallowed are sucked back through this outer tube before reaching the stomach.

The tube (nelaton catheter) can be swallowed by most people without difficulty and retained indefinitely with no retching and little or no salivation. Since this stomach tube cannot be pushed into the stomach it cannot be used in refractory individuals (insane, hysterical subjects), and because of its diameter, the tube cannot be used to recover gastric contents containing foods not thoroughly masticated.

The scientific value of much of the experimental and clinical data on gastric secretion is greatly decreased by the failure to secure controls on the continuous secretion.

*Extracts of the gastric gland.* The study of gastric secretion by extracting the mucosa is of very limited value, except as an aid in localization of the pepsin-producing glands. So far, this method has yielded nothing conclusive on the question of place and mode of origin of the acid and ferments of the gastric secretion.

*2. Method of determination of gastric acidity.* The literature on methods of measuring the acidity of gastric juice is considerable.

The direct titration with NaOH was started by Sazabo, and developed into the present form (using dimethyl-amin-azo-benzol) by Köster, Mintz, Mörner and Tröpfer. The controversy concerning the method has been as to which of the numerous indicators used yields the most accurate result, and whether the figures for free HCl are not too high, the addition of the alkalis liberating some of the combined acid. To overcome this last objection Sörenson introduced the colorimetric method. The principle of this method is the selection of a series of indicators that change in color at definite degrees of acidity. Sholl has recently worked out a series of indicators and stable color standards permitting direct reading of the approximate pH of the gastric juice.

Theoretically, the gas chain method yields the most accurate information of the true acidity of a solution, and this method has been used by a number of investigators on the gastric juice (Michaelis and Davidson, Christiansen, Menten, McClendon). McClendon has devised a hydrogen electrode which can be introduced into the stomach or duodenum by means of a catheter. This permits the determination of gastric and duodenal acidity during digestion without removing any of the gastric content. Christiansen has reported a thorough-going comparative study and critique of these several methods of determining gastric juice acidity. On normal human gastric juice the standard titration and the gas chain method yield figures so nearly identical that the titration method is sufficiently accurate for all clinical and for most research purposes.

3. *Methods of measuring the pepsin concentration.* Since the chemical structure of pepsin is not yet known all methods of determining the pepsin concentration in gastric juice are only relative. The methods are all based on the rate of solution of some protein. Grützner modified the older method of Brücke by using carmine-stained fibrin, the rate of digestion being measured by the rapidity of liberation or solution of the carmine. Jacoby used the rate of clearing of a solution of ricin as a measure of pepsin concentration. Fuld and Levinson used edestin. Gross used casein, Hammarschlag egg albumen, the rate of digestion being determined in each case by the amount of protein remaining to be precipitated by sodium chloride, sodium acetate and Esbach's reagent, respectively. Huppert and Schütz used the rate of production of secondary albumoses as a measure of pepsin, and on the basis of this method they formulated the law that the rate of pepsin digestion is proportional to the square root of the pepsin concentration, a generalization that has, at the most, only a limited application (Oppenheimer). Glässner uses the rate of hydrolysis of globin.

The simplest, and for clinical purposes at least, quite the most satisfactory method is that of Mett, the pepsin concentration being calculated from the rate of digestion of egg white coagulated in glass tubes. Pepsin concentration has also been measured by the changes in the viscosity (Spriggs), and the electrical conductivity (Sjöquist, Oker-Blom) of protein solutions.

II. *Secretion of gastric juice in the lower animals: 1. Gastric secretion in the invertebrates.* Gastric juice, with its free hydrochloric acid and specific ferments (pepsins) appears to be confined to the vertebrate animals. No free mineral acids or proteolytic ferments requiring free acids for their digestive action have so far been conclusively demonstrated in the stomachs of any invertebrate group. Acid reaction of gastric contents or secretion have been noted by many observers, and some have assumed this to be due to hydrochloric acid. Later and more careful work seems to show that this acidity is due either to *acid salts* or to acid saliva, and not to acid gastric juice, although Griffith quotes Fredericq as having reported that the gastric juice of *Mya* contains hydrochloric acid. Bodansky and Rose studied the digestive action of gastric mucosa extracts of certain coelenterata (*Stomolophus*, *Physalia*) on gelatin. They conclude that pepsin is present, since the extract liquefied gelatin in an acid medium, the optimum acidity being pH 3.0, or the same as for mammalian pepsins.

The free mineral acid ( $H_2SO_4$ ) in the so-called salivary glands of some molluscs is not accompanied by pepsins or pepsin-like ferments. The digestive significance of this salivary mineral acid is therefore not analogous to the hydrochloric acid of the vertebrate gastric juice. There appears to be, at times, free mineral acids in the digestion vacuoles of some of the unicellular animals, but in these animals no proteolytic action goes on until the free mineral acid is fixed or neutralized. The rôle of this acid vacuole secretion is therefore supposed to be the *killing* rather than the *digestion* of the ingested bacteria and spores. The literature is adequately reviewed by von Fürth, Griffith, and Jordan. Invertebrate digestion is essentially pancreatic and intestinal.

The biological significance of this apparently uniform absence of gastric secretion (HCl, pepsins) in the invertebrates, and its normal presence in the entire vertebrate phylum is by no means clear. But it would seem that gastric juice is a comparatively late addition to the animal digestive process. It might be worth the while to reinvestigate the gastric secretion in the groups supposed to be the nearest relatives of the primitive vertebrates.

The late evolution of gastric juice may have some significance in the frequency of chronic achylia in normal persons and the apparent absence of digestive disorders in chronic or temporary achylia not complicated by motor disturbances of the gut.

There appears to be a continuous secretion of the digestive juices in most of the invertebrate groups. The question of nervous and chemical control of these secretory processes does not appear to have been investigated.

2. *Gastric secretion in the fishes.* There is a considerable literature on gastric secretion and gastric digestion in fishes, beginning with Spallanzani (1783). This is adequately reviewed by Sullivan and by Beidermann. The problems attacked are the nature of the acid or acids, and the proteolytic ferments, and the place of formation of the acids and the ferments. The question of the mechanisms of secretion of the gastric juice has scarcely been touched in the fishes, although some species of elasmobranchs would be suited for gastrotomy and Pavlov pouch operations. Sullivan concludes that in the elasmobranchs both the pepsin and the hydrochloric acid is secreted by the cells in the gastric sac, that is, the region corresponding to the fundus of the mammalian stomach. In the elasmobranchs the fundus glands are not differentiated into the chief and the parietal cells. According to Sullivan the pyloric mucosa secretes neither pepsin nor HCl.

It seems established that the gastric glands in all groups of fishes secrete HCl and pepsins. Some observers have also reported sulphuric and organic acids. In fact, Weinland and Van Herwerden report that the gastric juice of the shark contains only organic acids. But the gastric secretion in fishes seem to differ from that of the mammals in three particulars: *a*, the high concentration of HCl; and *b*, the rapid action of the fish pepsin at low temperatures; *c*, the high optimum acidity for the action of the fish pepsins. Richet reported a gastric acidity in selachians of from 0.6 per cent to 1.4 per cent. Young reported an acidity of 0.84 per cent. Sullivan reports that in the fasting shark the stomach reaction is practically neutral. His observations were therefore made on the gastric content at the height of digestion; under those conditions he found the total acidity as high as 1 per cent HCl, and the free or physiologically active acidity as high as 0.6 per cent HCl. A Russian observer (Svolima) has recently reported an acidity of 1.6 per cent HCl in the shark's stomach. This observer appears to have secured pure gastric juice by means of a gastric fistula. Wieland reports the secretion of an alkaline gastric juice in the skate (*Raja*).

The high acid optimum (0.5 per cent free HCl) and the rapid proteolytic activity of fish pepsins at low temperatures are interesting differences from mammalian pepsins that deserve more detailed investigation. Using artificial gastric juice (extract of mucosa) of fishes and ordinary commercial pepsin, Bodansky and Rose found the optimum for fish pepsins to be the same as for mammalian pepsins or pH 3.0. The reports are conflicting as to the presence of rennin and lipase in the fish gastric juice. Bodansky and Rose found rennin present in the gastric mucosa extract of some species of fish, absent in others. Some observers report a high acidity in the fasting fish stomach. This may be either appetite secretion or continuous secretion.

Riddle introduced Mett's tubes directly into the stomach of fishes and determined the variations in the rate of the gastric digestion with the season and the temperature. He reports that temperature and season being the same, the gastric digestion in fishes is much more rapid than in frogs and turtles.

3. *Gastric secretion in the amphibians, reptiles and birds.* Our knowledge of the gastric secretion in these animal groups is very fragmentary, despite the fact that the earliest experimental work on gastric secretion and digestion appears to have been done on birds (Reaumur, 1752).

Spallanzani found an abundance of clear acid (to taste) fluid in the stomach of chickens, geese and falcons.

Klug reported that the gastric mucosa of geese contained pepsin, and later Paria-Mall and Braimaier found that the pepsin content of the gastric glands is greatly reduced during digestion and rapidly restored in the first hours of fasting, despite the evidence of continuous secretion in the fasting stomach (Teichmann). The difference in the pepsin content of the fasting and the digesting gastric mucosa in birds appears much greater than in the mammal.

Pepsin appears to be absent from the mucosa of the crop, but proteolytic digestion may go on in the crop as a result of gastric juice being forced backward into this organ. The frequent presence of bile in the gastric juice of the empty stomach suggests duodenal antiperistalsis induced as an acid reflex similar to that in mammals.

Karpov made a gastric fistula in the goose and secured gastric juice by sham feeding. This gastric juice showed an acidity of 0.30 per cent HCl, but was very poor in pepsin. Collip reports that extracts of the chicken proventriculus and duodenal mucosa as well as extracts of the thyroid cause secretion of gastric juice in the chicken. In chickens with esophageal fistula, forced swallowing of water also induces gastric juice secretion. Kaskowski, working on pigeons provided with gastrostomy, states that histamine given by mouth or intravenously does not induce gastric secretion, while hypodermic or intramuscular injections of the drug are effective. Very large doses of histamine introduced directly into the intestine also induce gastric secretion.

Langley described the usual histological changes (decrease in granules) in the gastric cells of the snake during digestion. Langley obtained little or no pepsin from the mucosa of the pyloric region.

Swiecicki reported an abundance of pepsin in the esophageal mucosa of the frog, and this appears to be substantiated by Langley and others both by histological and biochemical methods. The granules of the gastric and esophageal cells are diminished during digestion, but the restoration of the granules begins before the gastric digestion is complete. The HCl appears to be secreted by the gastric glands only.

Riddle put Mett's tubes directly into the stomach of frogs, salamanders and turtles and found that the digestion rate was at the minimum in January-March (hibernating season), and at its maximum in midsummer. Riddle's results indicate a continuous secretion of gastric juice in the stomach during the hibernating season, as the method of investigation probably eliminated the appetite secretion factor, if this mechanism is present below the mammals.

The problems of nervous and hormone control of gastric secretion have not been adequately studied in these groups. Boenheim appears to have demonstrated secretion of HCl by the excised gastric mucosa of the frog under the influence of pilocarpin.

III. SECRETION OF GASTRIC JUICE IN NORMAL MAMMALS. *A. Contents of the "empty" stomach.* The normal stomach, empty of food, usually contains some fluid and mucus. This fluid of the empty stomach is made up of 1, gastric juice; 2, saliva; and 3, duodenal contents (bile, pancreatic juice and succus entericus). The chemistry of the contents of the empty stomach depends on the relative preponderance of these three factors. The duodenal juices are frequently absent from the empty stomach. If the continuous gastric secretion is unusually rapid, the empty stomach content may approach the composition of pure gastric juice.

The quantity of empty stomach content varies greatly even in apparently healthy persons. Verhagen reported as high as 50 cc. with an average of 10 to 25 cc.; Moritz found 24 to 64 cc.; Rehfuss, Bergheim and Hawk find 30 to 180 cc. to be within the normal range. Fowler and Zentmire found in ninety healthy women an average gastric content of 50 cc. The average of several hundred observations on the author's three gastric fistula cases (two adult men and a girl of twelve all having cicatricial stenosis of the esophagus) is 30 cc., with variations of 5 cc. to 120 cc. We have occasionally found normal subjects with the stomach literally empty in the morning before breakfast. We found on Mr. V. on the whole the content of the stomach greater in the morning before breakfast than at noon before lunch. It was also greater in the summer than during the winter months. These variations are probably related to gastric tonus and motility rather than to the rate of continuous gastric secretion.

The acidity of empty stomach contents may vary from almost zero up to full gastric juice acidity. In all three gastric fistula cases the figures ran as follows:

	FREE ACIDITY PER CENT			TOTAL ACIDITY PER CENT		
	Low	High	Average	Low	High	Average
Mr. V. ....	0.10	0.35	0.18	0.15	0.40	0.23
Mr. E. ....	0.09	0.36	0.20	0.20	0.42	0.25
Miss C. ....	0.08	0.40	0.22	0.13	0.45	0.26

The pepsin concentration of the empty stomach content may be higher but is usually a little lower than that of pure gastric juice.

*B. The continuous secretion of gastric juice by the fasting stomach in normal men and animals.* Beaumont and Pavlov appear to be mainly responsible for the view that in the absence of food or so-called psychic stimuli, the gastric glands are quiescent. The corollary to this, namely, that secretion of gastric juice in the absence of food or "psychic factors" is a pathological manifestation has had wide acceptance among clinicians. Both of these views are erroneous. It has been shown that a more or less continuous secretion of gastric juice in the absence of food and evident psychic factors is a normal phenomenon both in man and experimental animals. The complete rest of the gastric glands is an exceptional state in the healthy individual and does not occur for long periods, even in prolonged fasting.

We have made observations on three gastric fistula cases (normal persons except for cicatricial stenosis of the esophagus). On one of these subjects, Mr. V., the observations extended over six years. In this individual the continuous secretion varied from 10 cc. to 60 cc. per hour. A continuous secretion was practically always present also in the other two cases, but the observations on them were not so extensive. If the gastric juice is removed from the stomach every 10 or 15 minutes the total secretion for the hour is greater than if it is removed at longer intervals. This may be due to passage of some of the secretion through the pylorus.

The continuous secretion is true gastric juice, containing HCl and pepsins. The percentage of acidity varies directly with the secretion rate, but is usually less than that of appetite gastric juice.

It may be objected that persons with gastrectomy and cicatricial esophageal stenosis are not normal, since they have a rubber tube in the stomach cavity. But Pavlov has shown that mechanical stimulation of the gastric mucosa is probably not a stimulus to gastric secretion, and it is difficult to imagine how a cicatricial stenosis of the esophagus not involving the vagi might induce gastric secretion, except by reflexly increasing an existing vagus secretory tonus. Moreover, the presence in the stomach of a rubber tube or other collecting devices is necessary even for observations on the empty stomach of normal men and experimental animals.

Rehfuss, Bergheim and Hawk have reported many cases in which the gastric secretion continued for a half to one and a half hours after all the food (Ewald meal) had left the stomach. This secretion might be

explained on the basis of a slow absorption of gastrin bodies produced by the preceding meal or as due to intestinal reflexes acting on the gastric glands. These factors should be eliminated when the observations are made in the morning before breakfast, that is, 12 hours after the meal. When this is done in normal persons, using the Rehfuß tube, we practically never fail to demonstrate a continuous gastric secretion of relatively low acidity, even though the observation be continued for hours. The quantity is from 30 to 60 cc. per hour. The admixture with swallowed saliva is difficult to control; duodenal regurgitation is also a disturbing factor. But the continuous gastric secretion is so universally present in normal persons that controls must be run on this factor in all accurate studies of gastric secretion.

The continuous gastric secretion is usually also in evidence in dogs with a Pavlov stomach pouch. This is denied by Bickel and Rheinboldt, but it has been demonstrated in our laboratory in the several lines of study of gastric secretory factors in such animals, particularly if the animals are in good condition. At times the secretion appears to be mainly mucus and pepsin, free HCl being absent.

Bickel has demonstrated a continuous gastric secretion in the goat. This will probably be found true for all the ruminants. Schalk studied the secretion of a Heidenhain pouch of the true stomach of the goat. The secretion was continuous even in prolonged absence of food. Three 72-hour fasting periods were run. In the first period 17 one-hour records were taken with an average secretion rate of 7 cc. In the second period 25 one-hour records showed an average secretion rate of 8.5 cc. At the end of the third fasting period the secretion rate was down to 3.5 cc. per hour, but equally low rates were sometimes noted on days when the goat had a full supply of food. The acid and pepsin of the gastric juice on the fasting days showed no deviation from the normal.

It is clear, however, that accurate studies of secretory factors even in Pavlov pouch dogs must include controls on the continuous secretion. The term "continuous" should not be misunderstood in this connection. The secretion is usually not uniform in rate but the periodicity of the continuous secretion is less marked than the periodicity of the motor phenomena in the empty stomach.

*C. The continuous gastric secretion in prolonged fasting.* Pavlov reported that the secretion of gastric juice on sham feeding in a prolonged fast ceases after eight or nine days unless NaCl is given in the drinking water. If the NaCl is sufficient the fasting itself is reported as not materially influencing the quantity or the quality of the appetite gastric

juice. Boldyreff, using dogs with gastric and intestinal fistulae, found that after 24 hours of fasting the gastric glands begin to secrete an abundance of juice periodically, and after 3 to 4 days of fasting the gastric secretion becomes continuous and so abundant as to inhibit the motility of the fasting stomach. Carlson found in man, fasting for 5, 8 and 15 days, no significant change in the rate of continuous secretion.

The acidity of the continuous secretion showed on the whole a slight increase with the duration of the fast, and with this appeared a greater frequency of regurgitation of intestinal content into the stomach. This continuous secretion is greatly augmented over a long period on breaking the fast. This has recently been confirmed by Kunde on Pavlov pouch dogs. Sutherland studied the continuous secretion in fasting dogs provided with Paylov and Heidenhain stomach pouches. He reports persistence of the secretion, with some periodic variations in the secretion rate throughout the fast. He found a gradual decrease in the quantity of the secretion unless NaCl was given with the drinking water. Hess and Taylor have reported a continuous secretion of gastric juice in the newborn infant before ingestion of food. Taylor gives the quantity as 60 to 200 cc. in 24 hours. In the guinea pig there may be demonstrated a continuous secretion of gastric juice during late intra-uterine life (Sutherland).

Politzer reports marked secretion of gastric juice before the first feeding in 100 human infants. He suggests that swallowed amniotic fluid may be the stimulus to this secretion.

The mechanism or the cause of the continuous gastric secretion is unknown. It is probably not appetite or psychic secretion, as this term is ordinarily understood. It may be a subconscious secretory tonus of the vagi, in which case the secretion should cease on double vagotomy. But this is not the case. We may have secretagogues produced in the autodigestion of the gastric juice itself, as well as by the digestive and bacterial processes in the intestines. Jarno and Hik think that the secretion follows periods of gastric hunger contractions. It is well known, however, that the secretion may persist during the gastric hunger contraction period as well as during the period of motor quiescence.

Luckhardt and Johnstone have recently found that the continuous gastric secretion in man is augmented on the induction of hypnotic sleep. A similar augmentation will probably be found to occur in normal sleep. This seems to indicate an inhibitory tonus (via the vagi?) on the gastric glands, similar to that found by Carlson for the gastric motor mechanism. These recent observations of Luckhardt and Johnstone

seem to demand a re-investigation of inhibitory secretory nerves to the gastric gland, the existence of which Pavlov thought he had demonstrated.

*D. The appetite secretion of gastric juice.* 1. Active secretion of gastric juice in the dog on seeing food was recorded by Bidder and Schmidt in 1852, and in 1878 Richet observed gastric secretion in man on tasting various foods. But it remained for Pavlov and his pupils to work out in greater detail this secretory mechanism by his classical experiments on dogs. Subsequent to Pavlov's researches, a number of investigators have worked on men with gastric fistulae and stenosis of the esophagus, conditions that closely parallel Pavlov's experimental animals (Kaznelson, Hornborg, Umber, Bogen, Sick, Carlson, etc.). The following points seem definitely established for man, monkeys, dogs and cats.

1. Seeing, smelling and tasting of food induce gastric secretion, provided the state of hunger and appetite is present. It is therefore a conditioned reflex (Bogen, Bickel). Tasting the food appears to be the most potent stimulus in most individuals; seeing and smelling the food may be without effect in some persons.

2. The vagi nerves constitute the sole efferent paths of this reflex. But direct stimulation of the vagi nerves yields little or no secretion of gastric juice, possibly because of the presence of nerve fibers that inhibit the gastric secretion (Pavlov).

3. The mere act of chewing indifferent substances, and the stimulation of the nerve endings in the mouth by substances other than those directly related to food, causes no secretion of gastric juice.

4. Sham drinking of water on the part of the thirsty animal starts secretion of gastric juice (Carlson, Orr and Brinkman). This may be due to a close association of the cerebral processes of hunger and thirst. Thirst may also have an inhibitory action on gastric secretion due to the concentration of the blood, as well as to the subjective processes of the feeling of discomfort.

5. Appetite secretion of gastric juice seems to be absent in adult ruminants (Bickel). If this is true it is probably a secondary adaptation or loss during the growth of the individual. It would seem that the mechanism would be as essential or useful to the sucking calf as to the sucking infant. Appetite secretion of gastric juice in the adult ruminants may be induced by the second mastication only.

2. The latent period of the appetite secretion is about 4 to 5 minutes in the dog (Pavlov). In man the latent period is shorter, especially if

the gastric glands are in a state of relatively active continuous secretion (Carlson). In the dog after 24 hours' fast, sham feeding for 5 minutes may initiate gastric secretion lasting for 4 to 6 hours (Pavlov, Rosemann). In man the appetite secretion is more transitory, usually ceasing within 15 to 20 minutes after completion of mastication (Carlson).

3. The rate of the appetite gastric secretion varies directly with the palatability of the food, and the degree of hunger and appetite. The maximum secretion rate in large dogs is reported as 4 to 8 cc. per minute (Konowaloff, Rosemann). In an adult man Carlson found the appetite gastric juice secretion rate to be on the average 3.5 cc. per minute, the lowest being 1.5 cc. per minute, the highest 11 cc. per minute. There is probably a great variation in this secretion rate even among normal individuals.

The quality of the appetite gastric juice depends on the secretion rate, and is independent of the character of the food.

4. We have several reports on the secretion of gastric juice in man induced by hypnotic suggestion. This phenomenon involves, of course, the nervous secretory mechanism. Bennet and Venable (using the Rehfuess tube in hypnotized subjects) found that suggesting hunger, food and eating caused secretion of gastric juice, while suggesting nausea depresses the gastric secretion. Heyer aspirates the gastric juice in hypnotized persons by means of a Nélaton catheter, and reports that on suggesting the eating of a meal there is a marked increase of the gastric secretion. This is a kind of sham feeding. Heyer reports also that suggesting pleasant or unpleasant experiences depresses this gastric secretion. This depression is most marked with the unpleasant suggestion.

Luckhardt and Johnstone, in our laboratory, have found that suggesting the eating of the meal to suitable hypnotized subjects produces as copious gastric secretion as the mastication (and spitting out) of a similar meal when the person is awake. The gastric secretion induced by the suggestion of eating in the state of hypnosis is very transitory. Needless to say that in these experiments the continuous secretion factor was carefully controlled.

In this work Luckhardt and Johnstone discovered a fact that seems to be of fundamental importance in regard to the nature of the nervous control of the gastric glands. They found that the continuous gastric secretion is invariably increased by the state of hypnosis itself, and in the absence of all suggestions of food. This fact points to an inhibitory

nervous tonus acting on the gastric glands in the waking state, unless the inhibition should be central (central depression of the vagi secretory fibers). It is probable that normal sleep likewise increases gastric secretion, just as normal sleep increases the tonus and contractions of the stomach. According to Tomaszewski, there is a paralytic secretion or hypersecretion in the Heidenhain gastric pouch for the first 7 to 10 days. It will be recalled that the gastric vagi fibers to this pouch are severed. Bickel and Rheinboldt also describe a continuous secretion in the Heidenhain pouch but not in the Pavlov pouch. These new facts give an additional incentive for renewed investigation of the mechanisms of inhibition of the gastric glands.

5. The significance of the appetite secretory mechanism in normal digestion is not clear. Pavlov identifies it with appetite and argues that both appetite and gastric secretion are necessary for the initiation of normal digestion. Some of his experiments on dogs seem to support this prevalent view. However, double vagotomy in dogs does not seriously impair digestion, administration of alkali in man to the point of complete neutralization of the gastric juice acidity does not seem to impair digestion, and all the appetite secretion induced by the normal mastication of a meal in man may be removed from the stomach before admitting the food (via gastrostomy) without impairing digestion or delaying the emptying of the stomach (Carlson). If the mechanism is not necessary for health, it is at least a factor of safety. But the continuous gastric secretion is sufficient to initiate gastric digestion. This new aspect of the appetite gastric secretion minimizes the importance of therapeutic measures that are supposed to induce or augment the appetite gastric secretion.

E. APPETITE GASTRIC SECRETION IN THE NEWBORN. Practically all observers agree that at birth the gastric mucosa of mammals is sufficiently differentiated to secrete normal gastric juice, except possibly for a slightly lower acidity and ferment concentration. The prematurely born infant secretes gastric juice (Politzer). In dogs and cats the gastric glands respond to gastrin when the fetus is within a few days of term, and in the guinea pig there may be spontaneous secretion of gastric juice in utero (Sutherland). But the question whether in the newborn mammal the appetite gastric secretory mechanism is already perfected is still open. Cohnheim and Soetbeer report secretion of gastric juice in newborn pups on sham feeding and, on nursing dry breasts. Noetemann reported appetite gastric secretion in a  $3\frac{1}{2}$ -year old child with gastric fistula and stenosed esophagus. Bogen's negative results might

have been due to so-called psychic inhibition. Taylor's work on numerous human infants seems carefully controlled, and he concludes that the appetite gastric secretory mechanism is not developed or functional in the newborn. If this is correct there remains to be determined at what age this mechanism becomes functional in man, and work should be done to determine the first appearance of the mechanism in the lower animals. The appetite secretion mechanism in the newborn ruminant should be investigated, in view of the report that it is not functional in adult ruminants (Bickel).

F. THE SECRETION OF GASTRIC JUICE INDUCED BY FOOD IN THE STOMACH AND INTESTINES. 1. *Direct mechanical factors.* Supported by Beaumont's experiments on Alexis St. Martin, the view that the mechanical stimulation of the gastric mucosa by foods induced secretion of gastric juice was generally accepted. Pavlov's experiments on dogs seem, indeed, convincing enough in eliminating this mechanical factor. But I do not believe that the matter is definitely settled. To be sure, in man and most of the higher animals the cells that secrete the gastric juice (apart from the gastric mucin) are situated so far away from the main cavity of the stomach that mechanical contact of the solid food particles with these cells is out of the question. But this does not exclude the possibility of the food acting mechanically through reflex mechanism. This possibility is not even excluded by the fact that the Heidenhain and the Pavlov accessory stomachs secrete gastric juice when food is present in the main stomach, for it is very difficult to sever all nerve connections with these stomach pouches without interfering with the blood supply. Such mechanical stimuli as sand blasts, test tube brushes or glass rods applied to the gastric mucosa may not be adequate. They may, indeed, be violent enough to induce reflex inhibition of the gastric secretion.

Pavlov has advanced the view that mechanical stimulation by the food of the sensory nerve endings in the gastric mucosa may induce the conscious sensation of appetite and thus through a central reflex induce gastric secretion. Some experimental evidence is cited in its support, but the view is at the most a working hypothesis, with most of the work yet to be done.

2. *The action of water.* Heidenhain, and Pavlov and his pupils showed that water in the stomach excites the secretion of gastric juice under experimental conditions that seem to exclude the psychic or appetite mechanism. The fact itself has been confirmed both on man and experimental animals by a number of subsequent investigators.

The literature is reviewed by Ivy and Sutherland. These authors, working in our laboratory, have endeavored to determine the mechanism of this water stimulation. Sutherland found that water and salt solutions (iso-, hypo- and hypertonic) stimulate the gastric glands. Water introduced directly into the intestines stimulates gastric secretion but to a less extent than does the same quantity of water in the stomach. Water given per rectum has little or no effect on the gastric glands, but Heyer has recently reported that distention of the rectum by enemas causes a reflex secretion of gastric juice. This needs confirmation and analysis. Food in the alimentary canal increases the gastric response to water introduced in the stomach or intestines. Ivy found that there is great variation in the gastric response to water in different individuals, and that on the whole the longer the water remains in the stomach the greater the gastric secretion.

Walenko reports that intravenous injections of hypertonic salt solutions induce secretion in the Pavlov pouch, and depresses the secretion in the Heidenhain pouch, explaining the latter by direct osmosis, the former by osmotic stimulation of the nervous secretory mechanism.

The response of the gastric glands to intravenous injections of water and salt solutions is probably an instance of the general tissue action in controlling hydremias. It seems also probable that water in the stomach and intestines may augment gastric secretion by a more rapid absorption of secretagogues.

3. *The peripheral action of proteins, carbohydrates and fat.* Under experimental conditions excluding the appetite secretion, so far as this is possible, as well as the entrance of the foodstuffs into the intestines, Pavlov and his pupils reported that meat extracts and fatty acids induced gastric secretion after a long latent period (15 to 30 minutes). Proteins in bread, raw or coagulated egg white, and meats extracted by boiling caused practically no gastric secretion, a slight secretion appearing after a latent period of more than an hour, and explained by Pavlov as due to secretagogues developed in the course of the digestion of these substances. Pure carbohydrates induced no gastric secretion. Fats by themselves had no effect, but given with meat or meat extracts the fats depressed both the appetite and the local or "hormone" secretion. Fatty acids stimulate the gastric gland, but Pavlov stated that hydrochloric acid itself tends to inhibit the gastric glands, and he sees in this a kind of autoregulative mechanism of the secretion.

According to Pavlov and his pupils meat, meat extracts or digested egg white have very little effect when introduced into the duodenum,

and no effect when introduced into the large bowel or parenterally. The fats inhibit gastric secretion even when introduced into the duodenum but this inhibition is decreased by section of the vagi (Orbeli). These investigators also report that bile and duodenal secretion, introduced into the stomachs stimulate the gastric glands.

These facts, based on experiments on dogs, have in the main been confirmed by subsequent observers both on man and experimental animals. The clinical literature is conflicting in regard to the effects on the gastric secretion of nutrient enemata, the discrepancies being probably due to failure to control the continuous and the appetite secretion, and the uncertainties of the ordinary clinical examination of gastric content. The main effort in the field in recent years has been attempts to work out the mechanism of this apparent local action of some of the food substances on the gastric glands.

Paylov took the view that it was a reflex mechanism, there being efferent nerve endings of specific sensibility in the gastric mucosa. Popielski showed that we are not dealing with long reflexes, because the phenomenon persists after isolation of the stomach from the central nervous system. On the basis of this fact Popielski proposed the theory of local reflex mechanisms. This theory has so far neither been proved nor disproved, but it is at present relegated to the background by the *gastrin theory*. This theory was initiated by the work of Edkins, who thought he had demonstrated a specific gastric secretagogue in the pyloric mucosa. The gastrin theory postulates that substances either in the native foods or developed in the gastric digestion of foods, act on the pyloric mucosa in such ways that a gastric secretagogue is produced, this is in turn absorbed into the blood and acts on the fundic glands via the blood.

Now, what are facts? In the first place, the alleged fact that meat extracts, or the end products of gastric digestion of proteins fail to cause gastric secretion when administered parenterally seems to support the theory to the extent that it eliminates the possibilities of secretagogues or gastrins in the food itself. We said "alleged facts," because more recent work has shown that gastric secretagogues or gastrins can be obtained from a variety of plant and animal tissues by hydrochloric acid extraction. The same chemical procedure has shown their presence in the mixed foods of the ordinary meal. Frouin secured gastric secretion both on ingestion and on hypodermic injection of gastric juice. Kisseloff reports gastric secretion on intravenous injection of watery extracts of strawberry, lettuce and spinach. Eisenhardt secured secre-

tion in the denervated Pavlov pouch on subcutaneous injection of extracts of meat, digested bread, extract of spinach and normal (entire stomach) gastric juice. Injection of fundic juice gave no secretion.

But on the positive side of the theory it has been shown that Edkins' experimental methods were so faulty that nothing can be concluded from his results (Ivy). The work of Gross supports the gastrin theory in so far as he found that meat extract caused gastric secretion when brought in contact with the pyloric mucosa. Gross' findings are directly contradicted by Ivy, who used better experimental methods. Ivy isolated the antrum pylori in Pavlov pouch dogs, and united the stomach with the duodenum. These dogs remained in good health over long periods. Ivy reported that substances like gastric juice,  $\frac{N}{10}$  HCl, Koch's gastrin, dextrose, peptone, fresh meat extract and Liebig's meat extract when kept in contact with the pyloric mucosa for an hour or more, have no effect on the gastric (fundic) glands. This would be an *experimentum crucis*, completely overthrowing the gastrin theory in its present form, provided we could feel certain that the pyloric mucosa was normal. Ivy showed that some of the pyloric mucosa functions (e.g., secretions, absorption) remained normal.

The work of Popielski, Ehrmann, Ensman, Eisenhardt, Maydell, Tomaszewski, Kissiloff, Koch, Luckhardt, Keeton, Rogers and Fawcett and others has demonstrated that a gastric secretagogue can by suitable means be secured not only from the pyloric mucosa but from the mucosa of the entire alimentary tract, and from such organs as the liver, the thyroid, plant tissues, etc. This has recently been denied by Lim, on basis of acute experiments on dogs under chloroform anesthesia. Negative results on anesthetized dogs cannot be used to contradict positive results on Pavlov pouch dogs.

Luckhardt has recently shown that gastrin (or the gastrins) is not specific for the gastric gland, since it stimulates both the stomach and the pancreas. The pancreatic secretin of Bayliss and Starling likewise stimulates both the pancreas and the stomach. It seems highly probable from present data that the gastrins are artefacts developed in the decomposition of the foods, or in the extraction of the mucosa and do not represent physiological mechanisms. They are all without action when given by mouth.

The action of the gastrin on the stomach is decreased but not completely stopped by atropin. We have thus an antagonistic action similar to that between pilocarpin and atropin. The gastrins probably belong to the pharmacology rather than to the physiology of gastric function.

The gastrins act more effectively on hypodermic and intramuscular than on intravenous injection. There is some evidence that the gastrins are rapidly destroyed by the liver (Bickel, Djenah).

*The chemistry of the gastrins.* Popielski endeavored to prove that the gastrins (and secretins) were identical with the vasodilators, the gland activity being a secondary effect of the capillary dilatation. This view has neither been conclusively proved nor disproved. But Luckhardt has recently shown that vasodilator substances do not necessarily initiate gland action. Histamine is a general secretagogue, and Popielski has advanced the view that this is the active substance in organ extracts. The studies of Koch, Luckhardt and Keeton indicate that gastrin is an imidazol derivative, like histamine and pilocarpin. They were able to separate histamine and gastrin by chemical means, which seems to show that they are different substances.

The gastrin theory may be correct but all the evidence on which it is based appears to have been completely disproved. If the theory is correct, the real gastrin has so far eluded direct detection and the whole subject of how the foods in the gut cause secretion of gastric juice must be re-investigated. The possible reflex factors have been neglected in recent years. The inhibition of the appetite secretion in the Pavlov pouch by fats in the stomach or in the intestine can hardly be explained on any other basis than as a reflex inhibition. And if we have inhibitory nervous reflexes from wide areas of the gastro-intestinal mucosa to the fundic glands, it is not probable that excitatory reflexes are absent. Tomaszewski thinks the secretagogues of meat (gastrins) act on local reflex mechanisms or directly on the gland cells. The latter possibility is eliminated by the fact that all the gastrins are without action when given by mouth.

*Gastric secretion from foods in the small intestines.* According to Pavlov and his pupils, the foods that have a marked stimulating action on the gastric glands when in the stomach have little or no action when introduced into the intestines. These results have been contradicted, at least in part, by later investigators. Tomaszewski states introduction of peptones into the intestines causes secretion of gastric juice if the vagi are intact.

Ivy has recently studied this question with improved technique. The dogs are provided with a Pavlov pouch and a fistula of the duodenum, the jejunum being sutured to the pyloric end of the stomach. These dogs remain in good condition. Meat extract, gastric juice and hydrochloric acid induce secretion of gastric juice after a latent period

of 30 to 45 minutes. Digested meat and Leibig's meat extract are without effect. Butyric acid, alcohol, glycerine, soaps and spinach extract in the duodenum likewise induce gastric secretion. Glucose, olive oil and mustard oil are ineffective. Gastric secretion is induced by NaCl (10 per cent) but not by  $\text{Na}_2\text{CO}_3$ .

*The secretion of the glands in the pyloric antrum.* The gastric secretion discussed in the foregoing section is that of the fundus or cardiac end of the stomach. In the mammals (excepting the ruminants) the pyloric mucosa secretes neither HCl nor pepsin. The pyloric secretion is alkaline, and is not increased by the various foods and secretagogue factors that stimulate the fundic glands. The literature is reviewed by Ivy (1921).

4. *The influence of acids, alkalis, salts, food condiments, alcohol and bitter "tonics" on the gastric secretion.* (1) We have seen that acids (HCl, fatty acids, normal gastric juice) appear to induce gastric secretion acting both from the stomach and the small intestines. The action of alcohol seems also clear as to the fact itself in that, however administered (per os, per rectum or parenterally) it excites the gastric gland (Chittenden et al., Chiari, Zitowitsch, Kast). The mechanism of this action is not definitely worked out. It may be, in part, a central depression of the inhibitory tonus governing the gastric glands in the normal animal. If this is the case alcohol should have much less action on the Heidenhain pouch as compared to the action on the Pavlov pouch. Ehrmann's observations do not support this view.

(2) The present information on the influence of acids in the gut on the gastric glands is both scanty and conflicting. Ivy obtained no effect from acids in the antrum pylori, but acids in the duodenum caused some secretion in the stomach after a long latent period. Pavlov claimed that acids in the stomach tend to inhibit gastric secretion, and Carlson found in man that frequent removal of the continuous secretion yields a greater total secretion per hour than hourly aspirations. Pavlov's view seems to be contradicted by the copious secretion of gastric juice in pyloric obstruction in man.

(3) Pavlov and his pupil Chigin state that NaCl has little or no influence, while  $\text{Na}_2\text{CO}_3$  in the stomach depresses gastric secretion. Subsequent work in this field, mainly with the alkalis, has yielded very discordant results. Lönnquist, Rosenblatt, and Bickel found that NaCl stimulated gastric secretion. Most of the observers (Rosenblatt, Ehrmann, Chiari, Hirsheimer, Bickel) report a depression of gastric secretion by the  $\text{NaHCO}_3$ . Others class the  $\text{NaHCO}_3$  as a gastric stimulant.

Most of these observations were made on dogs with accessory gastric pouches. Observations on man using the fractional analysis have not yielded conclusive results (Crohn). Boyd, working on Pavlov pouch dogs in the author's laboratory, found that  $\text{CaCO}_3$  and  $\text{NaHCO}_3$  usually stimulate gastric secretion until the quantity of alkaline administered (1.5 gr. or more per kilo body in 2 hours) induces nausea and vomiting. This depresses the secretion. This dose corresponds to about 100 grams  $\text{NaHCO}_3$  given per os to an average adult person in the course of 2 hours. Boyd's experiments were carefully controlled.

The practical interest in the action of alkalis on gastric secretion lies in the extensive use of carbonated drinks, and in their extensive clinical use to control gastric acidity and hypersecretion, especially in gastric and duodenal ulcers. It would seem that we have been using gastric secretagogues to control and depress gastric secretion. Of course, if enough of the alkalis are given, and if they remain long enough in the stomach (there are reports in the literature to the effect that alkalis hasten gastric evacuation) the direct neutralization may more than counteract the secretagogue effects.

The mechanism of the salt and alkali action on the gastric gland activity has not been satisfactorily worked out, despite its practical importance. Ehrmann states that  $\text{NaHCO}_3$  and  $\text{NaCl}$  have no inhibitory action on the Heidenhain pouch, similar to that on the Pavlov pouch. If this is correct, it indicates a reflex action from the stomach of these chemicals. He also reports that fats, soaps, morphine and concentrated sugar solutions have no influence on the Heidenhain pouch secretion; hence the action of these substances on the Pavlov pouch must also be a reflex.

It is difficult to account for these discordant results except on the basis of defective control of such factors as the appetite and continuous secretion, water intake, thirst, and the physiological state of the experimental pouch. Salts or alkalis will, of course, change the concentration of the blood, influence thirst, and therefore the water intake. Sutherland found that intravenous injection of iso-, hypo- and hypertonic  $\text{NaCl}$  solutions stimulates the gastric glands. This is probably in part an effect of the plethora.

Since the alkalis alter the control of the pylorus to a certain extent, this factor must also be controlled. The absorbable alkalis may influence the availability of the chlorides in the gastric glands, and thus influence the  $\text{HCl}$  secretion, but this does not seem a probable factor, since the animal must be in chloride starvation for a long time before the  $\text{HCl}$  of the gastric juice is appreciably affected.

It is stated by some clinicians (personal communication) that administration of large doses of alkalis over a long period (weeks) may produce an achylia lasting for several weeks after discontinuing the alkalis. This is important, if true, and would indicate a direct injury to the gastric glands. This condition has not been produced under adequate experimental control.

(4) Coffee, cocoa (minus most of the fat) and chicory in the stomach are reported to stimulate gastric secretion. Tea has no effect or may act as a depressant. The alkaloid caffeine has a slight stimulating action both on the Heidenhain and the Pavlov pouch (Pincussohn, Bickel, Ehrmann). Coffee, tea and cocoa are watery extracts of plant tissues, and as such may contain the gastrin bodies. But this direct action of these beverages on gastric secretion is of little practical significance, since the water content and the taste are greater factors in augmenting the gastric secretion.

The common food condiments acting from the stomach have little or no effect on the gastric secretion (Rabinowitch). The essential action of these substances is on the appetite secretion through taste. Raw onions, acting in the stomach, are reported to have a more pronounced secretagogue action than most vegetables (Wilbrand).

(5) Various bitter substances or bitter tonics have been used in medicine from time immemorial to aid gastric digestion. Do these substances increase the secretion of gastric juice? Pavlov and Borissow report that bitters given before the meal increase the appetite gastric secretion by increasing the excitability of the nerve endings in the mouth. Reichmann and Scheffer found that bitters in the stomach depress gastric secretion. Kaznelson and Bickel report that quinine in the mouth stimulates gastric secretion in man, but their experiments were not adequately controlled.

Carlson and Moorhead found that bitters had no appreciable effect on gastric secretion in normal man and normal dogs. In dogs rendered cachectic (experimental anemia), the bitters seem to have a slight stimulating effect on the gastric secretion, but this is too insignificant to have any real therapeutic significance. The value of bitters, if any, in human dietetics must be sought in other factors than in the augmentation of gastric secretion. Moorhead found that bitters given to cachectic patients may slightly increase the intake of food.

(6) Direct stimulation of the gastric gland with the direct or induced electrical current does not cause secretion of gastric juice. The x-ray and radium are also reported to be without effect, except for chronic

depression of the gastric gland when massive doses are used (Brenzel, Winternitz, Szegö).

(7) The secretion of the gastric mucin is a process largely independent of the secretion of water, HCl and ferments. All irritants, including mechanical and electrical stimulation, increase the mucin secretion, without increasing secretion of gastric juice.

5. *The reflex versus the hormone theories of the local gastric secretory mechanism.* (1) We have seen that the hormone or gastrin theory has not been disproved, but most of the data supporting it have been shown to be due to errors in technique or interpretation. What about the theory of local reflex mechanism as originally advanced by Pavlov? Some of the differences in the secretory response of the partially denervated Heidenhain pouch and the Pavlov pouch, which retains part of its vagi innervation, may be explained by absence of the secretory reflexes through the central nervous system, and by gradual deterioration of the glands in the denervated pouch. But I am not convinced that these are adequate explanations for all differences, despite the modern tendency to deny the existence of all local reflex mechanisms in the viscera. Many of the contradictory results are probably due to failure to control the continuous secretion and the vasomotor factors. The latter factor will probably influence the former. Unless the augmentation of the secretion in the Heidenhain pouch by the presence of food in the stomach and intestines can be explained on the basis of augmentation of the continuous secretion through circulatory changes, this would seem a strong support for the hormone or gastrin theory. Such secretion responses seem to obtain even after severance of the continuity of the Auerbach and the Meissner plexuses, but vasomotor reflexes (and possibly secretory reflexes) may go on via the nerve fibers along the blood vessels. Rheinboldt reports that the result of section of the nerves along the blood vessels to the Heidenhain pouch reveals some secretory function of those nerves, but his data are not conclusive.

(2) In normal men and animals all painful stimuli cause some inhibition of the entire phase of gastric secretion. Pain in certain pathological states in man (e.g., gastric and duodenal ulcer pains, pains in intestinal obstruction, etc.) seem to constitute an exception to this rule. Depressant emotions (fear, anger, anxiety, etc.) also depress both the appetite secretion and the local secretion phase. Strong emotions of pleasure seem also to cause some depression of the gastric secretion. Hence gastric secretion may be depressed from changes in the brain, from painful stimuli to the skin, and from certain types of stimuli (e.g., fat)

to the mucosa of the alimentary tract. The relative rôle played in this inhibition by stimulation of inhibitory secretory nerves, vascular changes in the stomach, changes in gut motility, changes in gastrin absorption, and changes in the activity of such remote organs as the adrenals and the thyroid, remains to be worked out.

The action of drugs on gastric secretion, such as the stimulation by pilocarpin and nicotin (Ehrmann, Skaller) and the depression by atropin (Keeton, Luckhardt and Koch) indicate but do not prove the preponderance of the nervous mechanisms. It seems significant, however, that atropin will completely inhibit the secretion from food in the stomach and intestines, while it decreases but does not completely inhibit the secretion from histamine and the gastrins. Adrenalin and pituitrin depress gastric secretion (Hess and Gundlach, Pal). This may be a vasomotor factor.

6. *Quantitative and qualitative adaptation of gastric secretion to the food.* (1) The appetite gastric juice is only quantitatively related to the food, in that, other things being constant, the more appetizing the food the more copious the secretion. This is true even of foods that do not require digestion or cannot be digested in the stomach. Arrhenius has attempted to show that there is a mathematical relation between the quantity of the ingesta and the quantity of the gastric juice produced. But the most important contribution to this subject has come from Pavlov's laboratory (Chigin). Thus Pavlov distinguishes between "meat juice," "bread juice" and "milk juice" as follows:

	SECRETION PERIOD	SECRETION TOTAL	ACID CONCENTRATION	PEPSIN CONCENTRATION
Meat.....	Medium	High	High	Medium
Milk.....	Short	Low	Medium	Low
Bread.....	Long	Medium	Low	High

According to Pavlov the stomach secretes more than double the quantity of pepsin for bread proteins than it does on equal quantities of meat or milk protein. These apparent qualitative adaptations of the gastric juice to the foods are given teleological interpretation by Pavlov. It is argued, for example, that the low acidity and high pepsin concentration of the "bread juice" is advantageous for the digestion of vegetable proteins.

- Assuming that the above facts are as stated (Arloing, Cade and Bocca) they seem to demand *a*, a relative independence of the processes of water, HCl and pepsin production by the gastric glands; *b*, specific

sensibilities of the afferent path in the reflex secretory mechanisms in the gastric and intestinal mucosa as assumed by Pavlov; or *c*, different kinds of gastrins (secretogogues) in the food acting with different intensities on the three secretory processes. In some animals at least the HCl and the pepsin appear to be secreted by different gland cells, and there is other evidence that the secretion of HCl and pepsin are more or less independent processes, but the other necessary elements of the theory (specific sensibilities of the reflex mechanism, or specific gastrins for each food) are as yet imagined entities.

The fact itself (the qualitative adaptation) should be reinvestigated in light of the more recent work on the gastric glands, and gastro-intestinal motility as influenced by the character of the food. In view of the variations in the gastric response to the same meal from day to day in the same animal, the original data of Chigin do not appear to me conclusive; and there is a seeming discrepancy between the rapidity with which a predominantly carbohydrate meal like bread leaves the stomach (Cannon) and the prolonged duration of the gastric secretory response to bread. There is some evidence that the acidity of the gastric juice increases with the secretion rate, while the pepsin concentration may actually decrease with the secretion rate (Carlson). This factor alone may explain the qualitative difference between the "meat juice" and the "bread juice" of Chigin.

7. *The composition of normal gastric juice.* (1) We have many analyses of pure gastric juice of the dog (appetite and digestion juice) and man (appetite juice), the latter secured from gastric fistula cases. The data from various observers are in essential agreement, and there appears to be little or no difference in composition of pure gastric juice in dog (Rosemann) and man (Carlson). Normal human gastric juice has the following composition:

Acidity	Free HCl = 0.40-0.50 per cent
	Total acidity = 0.45-60 per cent
Solids	Organic = 0.42-0.46 per cent
	Inorganic = 0.13-0.14 per cent
Specific gravity = 1006-1009	
Osmotic concentration = $-0.55^{\circ}$ - $0.52^{\circ}$ C.	
Total nitrogen = 0.051-0.075 per cent	
Amino acid nitrogen = 3-10 mgm. per 100 cc.	
Ammonia = 2-8 mgm. per 100 cc.	
Chlorides = 0.50-0.58 per cent	

The concentration of the ferments and the mucin cannot at present be expressed in percentages.

Bacteria are present in normal gastric juice both in man and dog. The antiseptic action of gastric juice has been known since the days of Spallanzani, but the bactericidal power of gastric juice is generally over-estimated. Burget found 25,000 to 100,000 organisms (yeast, bacilli, cocci) per cubic centimeter of normal human gastric juice (full normal acidity and peptic power). Poppens found *B. coli*, staphylococci and non-hemolytic streptococci in the gastric juice of dogs.

(2) The gastric juice secreted at a rapid rate has a higher acidity than that secreted at a slow rate. This is probably due in part to neutralization by gastric mucin, and formation of ammonia from the protein of the gastric juice, when saliva and regurgitated duodenal juice are excluded. This is indicated by the fact that the total chlorine of the slowly secreted juice is not as low as would be indicated by the low acidity. But there appears also to be some actual increase in acidity with the increase in the secretion rate (Carlson, Rosemann).

The evidence in favor of the view that the HCl is produced by the "border cells" of the gastric glands seems fairly conclusive, but we do not know how or where these cells produce it. In fact, there is some evidence that the acid may not be produced as such within or even on the surface of the cells. Using the Prussian blue reaction originally tried by Claude Bernard, Fitzgerald reported that the HCl was produced within the parietal cells. After showing the unreliability of the Prussian blue reaction, Harvey and Bensley concluded, on the basis of a different stain reaction, that the HCl first appeared in the lumen or neck of the gland at considerable distance from the parietal cells, while Collip appears to have proved that the HCl is formed on the cell border. The discrepancies in the results obtained so far by the staining methods are probably in part due to the condition of the gland cells when the stain is applied and the cells actually seen. From what we know of gastric secretory activity, it seems highly probable the cells observed by these investigators were not actively secreting, but were dead or moribund. Experiments bearing on this question are not physiological, unless the cells are secreting gastric juice when observed, or unless the chemicals used in the reaction are known not to impair the secretion process at least before they reach the interior of the cells. Unfortunately, the smaller transparent invertebrates, where one can observe the gastric mucosa under the microscope in the living animal, do not secrete an acid gastric juice. But it is possible that sulphuric acid secreting salivary glands in some molluscs is a better object than the vertebrate stomach for the solution of the problem how living cells produce in-

organic acids in relatively strong concentration. The high concentration of the HCl in the gastric juice has been cited as an argument against the production of the acid within the cells, as such high concentration of a strong acid would kill the cell itself. This point does not seem well taken. The gastric juice in contact with the surface of *living cells* does not kill all cells. This is true not only for the mucosa of the alimentary tract, but for organs not "acclimatized" to gastric juice, like the spleen and the kidney (Dragstedt).

The secretion of HCl in the gastric juice is not intimately dependent on the chlorides of the food, because complete chloride starvation must be maintained such a long period before the HCl of the gastric juice disappears (Wohlgemuth, Rheinboldt, Takata, Rosemann). In chloride starvation the gastric glands can apparently produce HCl until the secretion itself ceases from general cachexia.

The theory of Harvey and Bensley that parietal cells secrete the HCl as an organic or colloid compound demands investigation, if it should be shown that the acid is not liberated in or on the surface of the cells. Rosemann has suggested an actual secretion of lactic and phosphoric acids by the gastric glands under certain conditions.

(3) The origin and possible significance of ammonia of the gastric juice has recently been investigated by Huber on man and dogs. He found marked but consistent individual variations in apparently healthy subjects. The gastric juice ammonia is increased by a high protein diet and by adding ammonium salts to the food. This indicates an excretion from the blood. The fundic mucosa contains more  $\text{NH}_3$  than either the pyloric or the cardiac mucosa. This may indicate a relation of the gastric juice ammonia to the hydrochloric acid secretion. The bacteria of the gastric juice may also be a factor. The gastric juice ammonia appears to have no pathological significance, as the concentration may be as high in normal individuals as in patients with gastric ulcer and gastric cancer.

(4) The most important ferments of the gastric juice are the pepsins. During relative quiescence of the gastric glands the pepsins in an inactive form (pepsinogens) are stored up, possibly in the form of granules, in the chief cells. The pepsinogens are activated by acids. This may be one significance of the differentiation of HCl and pepsinogen production in separate cells. Lipase is also present in pure gastric juice (Davidsohn, Hull and Keeton). This lipase appears to be an actual secretion of the gastric gland rather than a diffusion product from the blood and lymph, as it appears to be present in much higher concentration in

the gastric juice. The physiological significance of the gastric lipase is obscure, as it is quickly destroyed by the acidity of normal gastric juice.

The long controversy as to the identity of the pepsin and the rennins seems to have ended in favor of Hammarsten's view that they are distinct groups (Taylor, Levy, Burge, Hammarsten). If this is correct the biological significance of the rennins becomes a riddle, as they are present in the gastric secretion from the fish up, that is, in animals where milk is absent from the diet.

IV. THE SECRETION OF GASTRIC JUICE IN DISEASE. The literature on the pathological physiology of gastric secretion is very voluminous, also very conflicting, not so much in the facts reported as in the significance in the disease complex assigned to the altered gastric function. The conflicting interpretations are largely due to the fact that we did not know until recent years what degrees of variations in gastric secretion are found in apparently healthy, and hence presumably normal, persons, and the further fact that the clinician very seldom knows anything of the gastric secretion in the patient before the onset of the particular ailment that brings him to the doctor's office. But despite this uncertainty as regards the "normal standard," and the usual lack of "controls" on the individual patient, the clinical and experimental literature yields today fairly conclusive answers to the following questions:

1. What are the variations in gastric secretion in normal individuals?
2. Are there primary hypo- and hyperfunctions of the gastric glands?
3. What diseases, not primarily involving the stomach, induce changes in gastric secretion?
4. Do primary changes in the gastric secretion itself produce disease?

A. *The variations in gastric secretion in normal individuals.* If one studies a large group of persons, otherwise normal, one encounters all the variations in gastric secretion seen in the sick (Martin, Rehfuess and Hawk, Bennett, Bennett and Dodd, Best, Alsberg, Sailor), except possibly the degree of hypersecretion associated with obstruction (functional or anatomic) at the pylorus. Thus one finds in normal individuals hypersecretion and so-called "hyperacidity," and various degrees of hyposecretion or hypoacidity down to complete achylia. One may question the correctness of calling such persons "normal," but they are normal at least to the extent of showing few if any symptoms referable to the change in the gastric secretion. The clinician must henceforth take cognizance of the fact that hypersecretion and clinical hyperacidity as well as hypo- and anacidity are not only compatible

with health, but are found in a considerable percentage of normal individuals. The anomalies in gastric secretion (except gastric retention) exhibited by a patient with any disease may therefore have been present in that individual before the onset of the malady. That complete absence of gastric juice should cause no evident digestive disorder may seem contradictory in view of the important rôle usually ascribed to the gastric juice in the functions of the gut. The explanation is probably to be found in some compensatory mechanisms.

The anacidity may be present without absence of gastric ferments, but usually there is a decrease in pepsin parallel with the hypochlorhydria.

*B. The etiology of primary hyposecretion and achylia gastrica simplex.* Some cases of achylia have been described as congenital and possibly hereditary (Dauwe), but this seems at present little more than speculation. Others look for the cause in permanent injury to the gastric glands during the life of the individual from such agencies as bacterial toxins, alcohol, too hot or too coarse food, poor teeth, chronic inhibition, etc. (Ramond, Faber, Levinson, Williemse). According to Rosemann there is less storage of chlorides in the gastric mucosa and more chloride salts in the gastric content in persons with hypo- and anacidity. Achylia seems to be more common in women than in men. That the condition is either hereditary or that the injury to the gastric glands is irreparable seems to be indicated by the usual permanency of the condition. This would seem to exclude nervous inhibition as a factor. Leist claims that hypo- and anacidity are associated with a lowered concentration of the blood proteins.

*C. Gastric hyposecretion and achylia associated with extra gastric diseases.*

1. *Cancer*, irrespective of the location of the tumor, is associated with hypo- and anacidity more or less in direct proportion to development of general cachexia. The hypoauidity usually appears, therefore, late in the cancer history, but there is much individual variation in this regard, indicating that the degree of general cachexia is not the only factor (Palmer). Possibly the initial vigor of the gastric glands may explain this variation. The gastric secretion does not seem to be influenced by the transplanted tumors in rats (Copeman and Hake). Moore advanced the view that the hypoauidity of the gastric secretion in cases of malignant growth is due to an excess alkalinity of the blood. The real explanation is probably more complex.

2. *The anemias*, pernicious, secondary, and experimental, are practically always associated with gastric hypoauidity and anacidity parallel with the degree of cachexia. Hypoauidity is also present in infantile

anorexia and atrophy (Wentworth, Sauer), edema, and very frequently in diseases of the gall bladder (Behm, Rydgard, Rhode, Blackford), in chronic colitis, and in marked hypothyroidism (Boenheim). The gastric hypoacidity may persist after removal of the diseased gall bladder. The hypoacidity is usually associated with increased gastric motility or at least a hastening of gastric evacuation (Voegler). Achylia appears to be induced in some cases by primary disturbances of the heart (Roemheld). Hypo- and anacidity are present in the pregnancy toxemias (Kramer-Petersen). The mechanisms producing the hypoacidity in these conditions are probably varied. Actual asthenia of the gastric glands is no doubt a factor, but bacterial toxins and persistent inhibitory reflexes may also play a rôle. Very little has been done to determine the etiology. It seems fairly certain that the hypoacidity is an effect of the diseases, not a causative factor.

Hypoacidity also appears in pellagra, and in beri-beri in man and animals (Kitamura, LaRue). It probably is one of the effects of all dietary deficiency diseases when the general cachexia is sufficiently advanced.

3. A number of clinical observers (Fleicher and Müller, Einhorn, Strauss, Woelpe) introduced the conception of a "dilution secretion" or "hydrorrhea gastrica" to designate what appears to be an increased volume of gastric juice parallel with hypo- and anacidity, if not actual alkalinity of the gastric juice. This may be seen in rare cases of gastric and duodenal ulcers, but is most frequently observed in cases of acute dilatation of the stomach. Dragstedt has recently shown that the large volume of fluid (usually alkaline) found in the stomach in the latter condition is not secreted by the stomach but comes from the duodenum. The case reported by Einhorn (gastric cancer with cirrhosis of the liver) may be simply a transudate due to partial obstruction and venous stasis. There may also be instances of actual transudate or exudates from the inflamed mucosa in ulcer regions. The case for a real "dilution secretion" or "hydrorrhea gastrica" has not been proved. The excess secretion of the duodenal and the gastric glands in paralytic ileus and acute dilatation of the stomach appears to be due to toxic amines or gastrins absorbed from the gut (Dragstedt).

4. *Depression of gastric secretion in fevers.* Following Beaumont, clinical observers are in practical agreement that in fevers the deviations from the normal gastric juice are in the direction of hypo- and anacidity. The same has been demonstrated in Pavlov pouch dogs (toxic fevers, thermal fever) (Meyer, Cohn and Carlson). The increased body tem-

perature itself, apart from possible action of bacterial toxins, depresses the gastric glands.

Great increase in the external temperature but without rise in the body temperature is reported to depress gastric secretion and acidity in man, in proportion to the profuseness of the sweating (Cohnheim and Kreglinger, Fischer). There are probably several factors involved in this gastric depression, such as concentration of the blood and inhibition from the mental discomfort of external heat.

5. Hemmeter reported that extirpation of the salivary glands in dogs led to a suppression of the gastric secretion. This has not been confirmed (Loevenhart and Hooker, Swanson). In fact the acidity of the gastric juice may be even slightly increased (but not above the normal limit) after removal of all the salivary glands.

A number of Italian observers have reported a depression (in pepsin) of gastric secretion after splenectomy, indicating some essential relation between the spleen and the gastric glands. These findings have not been substantiated (Inlow). The removal of the spleen decreases slightly the quantity of gastric juice, probably due to a slight interference with the circulation in the stomach.

Clinical observations after resection of the antrum pylori for gastric ulcer (Bilroth operation) indicate that the removal of the pars pylorica depresses the secretion of the fundic glands (see Babkin, Kelling). This needs experimental verification, and seems to be contradicted by the recent work of Ivy and Whitlow on dogs. It is possible, however, that the entire antrum pylori was not separated from the stomach in these experiments. Since extirpation of the pars pylorica hastens the emptying of the stomach, the operation probably shortens the secretion period.

C. *Alleged pathological "hyperacidity" and hypersecretion.* 1. It is now well established that in gastric and duodenal ulcers, uncomplicated by pyloric obstruction, we may have normal gastric acidity, so-called hyperacidity (that is, hypersecretion) or complete achylia. Since these secretory conditions are found in normal people, it is evident that gastric and duodenal ulcers do not *per se* alter the activity of the gastric glands. But in most cases of pyloric obstruction and consequent gastric retention, the secretory response to foods is prolonged, and there seems to be a tendency to excessive continuous secretion. Actual hyperacidity in the sense of a gastric juice of greater than normal acidity has not been demonstrated in any disease, and probably does not exist. The pathological deviation in acidity is always in the direction toward anacidity.

But actual hypersecretion may exist, although we have no accurate measure of the total gastric secretion in normal persons in the course of a day. It is not less than 1500 cc. and may be double that quantity. When we have partial obstruction at the pylorus the existence of actual hypersecretion cannot be proved, except by some method of continuous drainage of the stomach by the stomach tube. Crohn and Reiss state that ulcer patients may secrete 30 cc. of pure gastric juice in 5 minutes. Even more rapid secretion or 55 cc. in 5 minutes (appetite juice) may be seen in normal persons (Carlson), but this is very rare.

The gastric hypersecretion in ulcer complicated with pyloric obstruction is in all probability due to the increased time of action (gastrin or reflex) of the food in the stomach. The ulcer may also increase the reflex excitability, and actually liberate gastrins and amines into the blood.

Gastric stasis may result from a variety of causes, such as anatomical or functional obstruction at the pylorus, primary gastric asthenia, chronic inhibition, etc. It has not been established that any of these factors can induce gastric hypersecretion, apart from the stasis of food and gastric juice in the stomach. Primary hyperirritability of the gastric gland cells and the local secretory nervous reflex mechanisms; the genesis of gastrin bodies in the autolysis and resorption of tissues, etc., must ever be kept in mind as possible factors. But progress in this field of pathological physiology of man demands greater attention to the continuous secretion and to the limits of its normal variation, as well as to the limits of variation in the acidity of normal gastric juice than has been the usual clinical practice of the past. The notion that gastric content showing 0.2 per cent HCl acidity is the acidity of normal gastric juice, and that 0.4–0.5 per cent HCl is "hyperacidity" should no longer be permitted to confuse the issue. What pure gastric juice (acidity: 0.4 per cent–0.5 per cent) may do in the way of inducing disease symptoms when it acts on sensory and motor mechanisms that are already abnormal is another matter.

In dogs gastric and duodenal ulcers may or may not induce digestive and continuous hypersecretion (Hardt). There is no hyperacidity.

The great amount of attention given in the clinical literature to gastric secretion in gastric and duodenal ulcers is due to the supposed rôle of the juice itself in the etiology and chronicity of the ulcer, the ulcer pains and the pylorospasm.

2. Gastric hypersecretion is frequently seen in appendicitis and visceral adhesions. This has been demonstrated experimentally in dogs

(McWhorter). There is no hyperacidity. Complete pancreatectomy in the dog leads to an excess secretion of gastric juice of normal acidity (Steinberg). Gastric hypersecretion has also been described in patients with toxic goiter (Boenheim), and other nervous abnormalities ("vago-tonia") but Hardt found that feeding thyroid extract to Pavlov pouch dogs decreased the gastric secretion. It is probable that the hypersecretion associated with nervous disorders is in part secondary to gastric stasis.

3. The main established facts in the pathology of gastric secretion may be summarized as follows:

1. In otherwise normal persons the gastric secretion may vary from hypersecretion through normal and down to complete anacidity. These variations by themselves do not therefore, produce disease symptoms.

2. In chronic disorders gastric secretion and gastric acidity are decreased on the whole parallel with the degree of general cachexia. The most important factor in this depression is probably the cachexia of the gastric glands.

3. There is no disease known capable of inducing true gastric hyperacidity. The pathological deviations in acid and pepsin concentrations are invariably in the direction of a decrease.

4. Essential hypersecretion (Reichmann's disease) probably does not exist. The factors definitely known to induce hypersecretion are delayed gastric evacuation from obstruction at the pylorus or gastric stasis due to factors that do not at the same time depress the gastric glands. The hypersecretion that is frequently seen in certain so-called nervous disorders has not been sufficiently studied in regard to gastrointestinal motility. If this hypersecretion is primarily of nervous origin, it may be due to depression of the inhibitory secretion tonus quite as much as to excess activity of the appetite nervous mechanism. The marked hypersecretion following a prolonged fast does not seem to induce symptoms of disease.

5. The gastric juice (in normal or greater than normal quantities) can itself produce anatomic or functional disorders only when it acts on tissues or mechanisms that are already pathological.

6. The rôle of the gastric juice in the maintenance of health and in the etiology of disease has been exaggerated, to the neglect of the importance of normal gastric motility.

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## CELLULAR IMMUNITY: CONGENITAL AND ACQUIRED TOLERANCE TO NON-PROTEIN SUBSTANCES

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It is now well known that, when acquired immunity is induced in warm-blooded animals to toxins, bacteria, or indeed to any protein substance, this immunity is accompanied by, and at least partly due to, the formation of specific antibodies in the blood or tissue fluids. No such antibodies are formed to non-protein substances, though to some of the latter a high degree of immunity can be acquired. This fundamental distinction has been emphasized by the retention of the word "immunity" as applied to proteins, and by the restricted use of the word "tolerance" as applied to non-protein substances. While this difference in nomenclature is convenient and in part salutary, there is a danger that the distinction may overleap itself and fall on the other side; and this for two reasons. In the first place it has tended often to lead to a tacit and unjustifiable implication that the processes whereby an animal acquires tolerance to non-protein substances do not occur in immunity to proteins; and, secondly, the distinction does not hold good for congenital immunity, because there is no doubt that congenital immunity both to protein and non-protein substances is in many, and possibly in most, cases due to an insusceptibility of the cells themselves to the toxic action of the substance, and independent of any antagonistic effect of the body fluids.

While, therefore, it will be possible in the space of this review to deal only with tolerance to non-protein substances, the object of it is a twofold one. The subject of tolerance to drugs possesses its own interest and importance, but any considerable review of the whole subject has not to my knowledge been attempted since that by Hausmann in the *Ergebnisse der Physiologie* in 1907. This resumé deals largely with investigations subsequent to that date, and in the necessary selection of material I have chosen rather to consider in some detail only those instances of tolerance which have received some explanatory investigation, than to attempt to mention every known instance of tolerance. The primary object of this review is therefore to give a

general survey, from a scattered literature, of the present state of knowledge of tolerance to drugs. But, in addition, it may serve a purpose of providing a brief statement of the methods, other than by antibody formation or phagocytosis (neither of which has suffered from lack of attention) whereby an organism can acquire immunity, for it is conceivable that even in the case of genuine "immunity" these methods of defence will be found to play a more important part than has hitherto been realized. In any case perhaps this introduction will help to justify the title of the article, for it will be seen that tolerance to drugs is dependent mainly upon properties or activities residing in the cells themselves. All immunity is of course ultimately cellular for antibodies are formed by the cells, so that even the expression "cellular immunity" is one only of convenience.

CONGENITAL TOLERANCE. It is well known that not only different species of animals but different individuals of the same species may show marked differences in resistance to the action of many drugs. This difference in resistance is usually measured by the dose per kilogram of animal that is required to produce a certain physiological or toxic effect—or usually a lethal effect, for death is an important and usually unequivocal end-point. With a great many drugs, e.g., most heavy metals, quinine, chloral, phenol, etc., the M.L.D. per kilogram is very nearly the same for all species of warm-blooded animals; on the other hand with some drugs the difference may be as much as 100 to 1 or more. When one species shows a high resistance as compared with the majority, it is regarded as congenital tolerance in the former; if a low resistance, congenital intolerance or hypersusceptibility. The determination of the causes of these differences is easier in proportion to the degree of difference, and, as the difference between species is usually much greater than the difference between individuals, it is naturally to congenital tolerance in species that investigation has so far chiefly been directed.

Though some progress has been made in explaining—what for medicine generally is perhaps the more important question—why individuals vary in resistance, e.g., as the result of variations in diet, etc., (1), the limited scope of this review will allow consideration almost exclusively of congenital tolerance in species, and even here there are few cases where the cause of this tolerance is yet known.

*Congenital tolerance of the toad to toad-poison, and of the toad, snake, and rat to digitalis glycosides.* That the toad was "ugly and venomous" had been known for centuries before Vulpian (1854) first subjected the

secretion of the toad's skin to scientific investigation. He discovered its toxic action on the heart and likened it to that of digitalis. He found that the toad is very resistant, though not completely immune, to its own poison and is also much more resistant than the frog to the toxic action of digitalis. Fornara (1871) found that the toad is also resistant to antiarin, and since then it has been found that the toad is resistant also to all other members of the digitalis group which have been investigated in this relation, e.g., strophanthin, apocynamarin and helleborein.

Later it was found that both the rat and the grass snake show, as compared with other animals, a very high degree of tolerance to members of this same group of glucosides. The problems relating to the immunity of the toad to its own venom, and of the toad, snake and rat to digitalis glucosides, are related problems and have often been investigated together. They will here be considered together and in some detail, as this type of congenital tolerance has been investigated with perhaps greater thoroughness than any other.

The immunity of the toad to its own poison and to digitalis glucosides is a specific tolerance, the toad having no comparable resistance to other poisons. Thus Heuser (3) found that the M.L.D. of a large number of drugs, e.g., caffeine, veratrin, chloral, etc., was practically the same both for the frog and toad, though the toad withstood rather larger doses of muscarine and physostigmine. Kobert (4) found that the toad's blood vessels, when perfused, were less affected than the frog's blood vessels, not only by toad poison and digitalis but also by barium. Particular interest attaches to barium because if the toad were resistant also to barium it would almost certainly imply resistance to a particular type of physiological action rather than to a particular type of chemical compound, because, though the actions of digitalis and barium are similar, there is no chemical relation between the two. Heuser (3) however failed to find any increased resistance (as estimated by the M.L.D.) of the toad to barium, and Clark (5) found no increased resistance of the grass snake to barium. In view of Kobert's statement I have repeated these experiments (6) and found that the toad shows no superior tolerance to barium. The M.L.D. is the same for both toad and frog, and the concentrations required to arrest the perfused heart or to produce constriction of the blood vessels is the same in both animals.

The increased tolerance of the rat, as of the toad, applies also to all members of the digitalis group that have been investigated, e.g.,

strophanthin, digitalis, squill. It may reach a high degree; e.g., Hatcher (7) found the M.L.D. of strophanthin for rats to be 100 times that for cats. Clark found the M.L.D. of strophanthin for the grass-snake to be 30 times that for the frog, and that this tolerance in this animal is not associated with any general tolerance to drugs that produce systolic arrest of the heart. From these experiments it is clear that the toad, grass-snake and rat show a singular congenital tolerance to the digitalis group of glucosides which resemble one another in their physical and chemical properties as well as in their physiological actions. The question arises as to what is the explanation of this tolerance.

From a variety of evidence it has become clear that the congenital tolerance of these animals is in every case largely, if not entirely, due to an insusceptibility of the tissues, especially the heart muscle, to the action of digitalis glucosides; and the chief steps by which this conclusion has been arrived at will briefly be summarized. Heuser (3) could find in the toad no evidence of defective absorption, increased destruction in the blood, or increased excretion such as could explain the increased tolerance of this animal as compared with the frog. Hatcher found that the increased tolerance of the rat to strophanthin was not due to difficulty of absorption. Seeing that congenital tolerance in the case of other substances has sometimes been found to be due to increased destruction in the blood and tissues, experiments to determine whether this is true in the case of the tolerance in question have been numerous. Hatcher could recover in the urine nearly all the strophanthin subcutaneously injected in the rat. Roger (8) and Hatcher and Bailey (9) both showed that the mammalian liver did not absorb any strophanthin when the drug was injected into the portal vein. Clark (5) found no destruction of strophanthin by the tissues of the rat or snake.

But the fact that this type of congenital tolerance is due to insusceptibility, in the tolerant animal, of the tissues upon which the drug acts is capable of the following more direct proof. Strophanthin kills by its toxic action on the heart alone. Fraser and Mackenzie (10) showed that the difference in concentration which is toxic for the heart as compared with other tissues of a given animal (frog) is very wide, and there is ample evidence to prove that when strophanthin is injected subcutaneously the heart stops before any other tissue is vitally affected. If the tolerance is due to a tissue insusceptibility, it ought therefore to be revealed clearly by perfusion of the isolated heart. Gunn (11) showed that it required about 30 times the concen-

tration of strophanthin (perfused in Locke's solution) to arrest the rat's heart as sufficed to arrest the rabbit's heart in the same time. As the M.L.D. for the rat was about 30 times that for the rabbit, no further explanation of the tolerance was needed. This was later established for the snake's heart by Clark, who also came to the conclusion that the tolerance of the snake is entirely due to an insusceptibility of the heart to the action of the drug.

The further explanation of why there is this quantitative difference in different animals must depend upon the fundamental method of action of strophanthin on the heart; and this is rather beyond the scope of this review. It may be stated, however, that according to Straub (12) strophanthin acts by altering the physical condition of the surface membrane of the cells, without entering into chemical combination with the cell constituents. Neither he nor Clark (5) could find anything beyond traces of strophanthin absorbed by the perfused heart. The tolerance of these animals to strophanthin must depend, therefore, upon some physical difference in the surface membrane of the cells of the tolerant animals as compared with other animals, and this difference is probably a quantitative one seeing that the same qualitative effects are produced in the heart of both groups of animals but only by different concentrations.

The question of the relation, if any, between the immunity of the toad to its own venom or to digitalis glucosides and the fact that the toad secretes a poison having a similar action has naturally attracted attention. Vulpian (2) considered that the insusceptibility of the toad to its own poison was due to "a sort of accustoming produced by continued molecular absorption of the toxic humour." Phisalix and Bertrand (13) found, by biological assay, toad poison in the blood of the toad and also ascribed the tolerance to self-immunization. This explanation was the more welcome when the modern lore of immunity developed. There are many facts which render this hypothesis suspect, and it may be worth while to examine those facts here as the problem is one of considerable interest for immunity generally.

Kobert (14) stated that the larva of the toad and of the frog are equally susceptible both to toad poison and helleborein and that this was explained by the fact that the venom is developed only in the skin of the adult toad, and that the larva, not having been immunized by absorption of poison, is just as susceptible as the frog larva. I have not found any details of his experiments or of the stages at which the larvae were examined. The statement is certainly not true so far as

the reaction of tadpoles to strophanthin is concerned. I found (6) that the difference between frog and toad tadpoles is just as great as between the mature animals. Frog tadpoles were killed by 1 in 20,000 strophanthin within 24 hours, and by 1 in 100,000 within a week, whereas toad tadpoles continued to live, and indeed completed their metamorphosis, in a solution of 1 in 1000,—an astonishing and unexpected degree of tolerance, even in these circumstances, to a substance the M.L.D. of which for the adult frog was about half a milligram per kilo. If it is true that the poison is developed only in the adult toad, this goes far to disprove the assumption that the immunity of the toad to strophanthin is due to absorption of its own venom.

As I have pointed out elsewhere (11), the immunity of the rat to strophanthin cannot be explained by absorption of any known poison it secretes. Clark (5) found no body resembling digitalis in action in snake's blood and "the explanation of the toad's tolerance does not apply in the case of the snake." Though the grass snake shows an immunity to strophanthin (which acts physiologically like toad poison) the toad shows no increased immunity to snake venom (6). The analogy of the immunity of snakes to snake venom is illuminating. Snakes are highly immune to venom and this has always been regarded as due to self-immunization. But the reasoning is illogical for the grass snake is also highly immune to snake venom, the M.L.D. of cobra venom for the grass snake being over 100 times the M.L.D. for the frog; and yet the grass snake has no venom gland. It might be supposed that, though the grass snake has no venom gland, it might be self-immunized by some constituent in its blood allied to venom. But the cat shows also a high immunity to colubrine venoms (15), and even if it might be supposed to harbor some undiscovered secretion of the nature of a colubrine venom, it is hardly probable that it should also secrete a substance like a viperine venom, and yet the cat is also higher tolerant of viperine venoms (16). Moreover Abel and Macht (17) found that though the toad, *Bufo Agua*, is relatively immune to the digitalis-like constituent of its venom (Bufagin), it is not similarly immune to the epinephrin in its venom gland; and animals do not seem to become highly immune to their own internal secretions by continued absorption of them. Indeed, if the toad were to become immunized to the digitalis-like constituent of its venom by continual absorption of it, so far from this being a result so axiomatic as hardly to merit proof, it would seem to be an exceptional occurrence.

In short, that self-immunization is a necessary preliminary to congenital tolerance is clearly disproved by the occurrence of tolerance in animals which secrete no poison; that continual absorption of a poison does not necessarily lead to immunity is shown by the fact that animals do not become immune to their own internal secretions; the immunity of the grass snake to venoms shows that the immunity of venomous snakes to venom is probably not, or at least not solely, due to self-immunization. Heterodox though the opinion may be, it seems more in accordance with facts to suppose that, in the case of animals which secrete a poison and which show immunity to it, the congenital tolerance preceded the evolution of the poison secretion, and that possibly it was only in animals which possessed an inherent insusceptibility to a particular type of poison that the evolution of such a poison to a useful degree of development was possible.

*Congenital tolerance of the hedgehog to cantharidin.* The hedgehog shows a remarkable resistance to a variety of poisons, one of which is cantharidin. Ellinger (18) calculated that one gram of cantharidin is a fatal dose for 20,000 kgm. of man, 500 kgm. of rabbit, and 7 kgm. of hedgehog. He found that this tolerance was not due to defective absorption, for it was true also of intravenous injection. It was not due to chemical change or neutralization in the body because cantharidin could be recovered unaltered in the urine. His experiments showed clearly that the tolerance was due, partly at least, to an insusceptibility of the kidney cells to the action of cantharidin. For example, after an intravenous dose of 0.02 gram in a hedgehog—a dose which produced trifling effects—0.014 gram was isolated from the urine of 24 hours, whereas 0.0001 gram would produce a severe acute hemorrhagic nephritis in a rabbit. As in the case of strophanthus, so congenital tolerance of the hedgehog is due chiefly to an insusceptibility of the tissues in the tolerant animal to the action of the drug.

Many other instances of congenital tolerance are known, some of which will no doubt prove to be of the above type. Heuser (3) found that, to produce motor paralysis, a dose 100 times greater of *curare* is required for *salamandra maculata* than for *rana temporaria*. He was unable to confirm the statement of Phisalix that this was due to an antagonistic action of salamander serum. In this case tolerance may be due to tissue insusceptibility. But that congenital tolerance is not always due to this will be seen in the case of the tolerance of the rabbit to atropine.

*Congenital tolerance of the rabbit to atropine.* Buys (19) incubated emulsions of organs of frog, rabbit and dog with hyoseyamine and then extracted the hyoseyamine from the mixture. He found that the liver of the frog and rabbit completely destroyed hyoseyamine, whereas the liver of the dog did not. As the frog and rabbit were more tolerant of atropine than the dog, the superior destruction by the liver in the former animals pointed to an explanation of this tolerance, and this was the starting point for a series of observations on the power of the liver and other tissues to destroy alkaloids, a large number of these observations being concerned with atropine.

Cloetta (20), regarding the action of atropine as one chiefly on the nervous system, endeavored to determine whether, when atropine was injected, there was a difference in different animals in the amount of retention of it by the brain tissue. He used the rabbit (M.L.D. subcutaneously 0.5 gm. per kilo) as a tolerant animal compared with the cat (M.L.D. subcutaneously 0.03 gm. per kilo). He found no atropine in the brain of either and no difference in the rate with which atropine disappeared from the blood. Seeing that he, confirming Wiechowski (21), was able to recover only less than half of the injected atropine in the excretions, he concluded that atropine must be destroyed in the organism and endeavored to locate this destruction. Incubating atropine with liver and brain emulsions, he found that both, but especially the liver, could destroy atropine and that this power was more marked in the rabbit than in the cat or dog.

Fleischmann (22), following up older observations that a given dose of atropine produced more prolonged action in Berne rabbits than in rabbits from other districts and that this was due to degenerative enlargement of the thyroid common in the former animals, found that the blood (whole or defibrinated) or the serum, of normal rabbits could destroy atropine when left in contact with it *in vitro*, but that the serum of goitrous rabbits did not. Sera of other animals (fowl, calf, man) had not this destructive power. Later (23) he claimed to show that the serum of man destroyed atropine exclusively in cases of thyroid disease. Thyroid extract itself did not destroy atropine. Of the guinea pig, sheep, dog, cat, calf and fowl, only the serum of the first had any marked destructive action on atropine. He came to the general conclusion that the power of the blood to destroy atropine goes parallel with the natural resistance to atropine in different species and in different individuals.

Clark (24) found that in the frog the liver markedly, and the heart and kidney slightly destroyed atropine *in vitro* but not the blood (confirmed by Oettingen); that in the rabbit, the liver and serum destroyed atropine, but no other organ; that in the rat and cat, no organ destroyed atropine, not even the liver or serum.

Metzner (26) confirmed Fleischmann's observation that rabbit's blood could destroy atropine, but was unable to correlate the differences in resistance in individual rabbits with changes in the thyroid. He did agree, however, that the differences in individual resistance which were marked in rabbits did follow differences in individual destructive power of the blood. Danielopolu (27) found that the serum of a rabbit had a less intense action on the pure alkaloid than on the sulphate and that the serum of man, sheep and guinea pig had no action. Doblin and Fleischmann (28) showed that washed red blood corpuscles of rabbits had no power, corresponding to that of serum, to destroy atropine. Schinz (29) found great individual differences in different rabbits in the amount of atropine required to paralyze the vagus and a marked correlation between these differences and the differences in the destructive power of the blood.

Though there are discrepancies in these results, certain points common to them may be taken as established. The liver and plasma of the rabbit and the liver of the frog show a power to destroy atropine, which is not shared by these or other tissues of less tolerant animals. That the liver is more important than the plasma in this respect is suggested by the fact that it is the only organ which possesses this power in the frog—the most tolerant of all animals. Moreover, according to Schinz, to produce the same effect in the rabbit by injection into a mesenteric vein as by injection into an ear vein, a dose twenty times greater is needed. This, if true, would almost certainly imply that, even in the rabbit, the destructive action of the liver is more important, or at least more rapid, than that of the serum. It shows too that the destructive action of the liver, shown by various observers *in vitro*, is operative even in a higher degree *in vivo*. That destruction of atropine occurs *in vivo* in the rabbit, as also that it is an important factor in explaining the congenital tolerance of this animal to atropine, is shown, among other ways, by the experiments of Heffter and Fickewirth (30) who found that though the M.L.D. by subcutaneous injection is greater for the rabbit than for the dog, the M.L.D. by intravenous injection is the same in both. The tissues of both animals are equally susceptible to the toxic action of the drug, but, when the alkaloid is injected sub-

cutaneously, less atropine in an active form reaches those of the rabbit owing to the destruction of the alkaloid which goes on in this animal *pari passu* with absorption, but which does not take place in the dog.

While the general contention of these experiments has been to establish a parallel between congenital tolerance (in individuals and species) to atropine, and the power, peculiar or at least enhanced, which certain tissues in these more tolerant animals possess to destroy the alkaloid, it must be pointed out that, so far as our knowledge goes, the parallel is far from complete. Indeed this would perhaps have more clearly been realized but for a haziness of knowledge as to the relative degree of tolerance to atropine in different animals. An earlier estimate of this was given by Richet (31), and a later one by Clark (24), the latter being a table summarizing the M.L.D. for different animals as found by different observers. This has more recently been reinvestigated by Willberg (32) in a very complete series of experiments, and he has found the M.L.D. of carefully dried atropine sulphate by subcutaneous injection in grams per kilogram to be as follows: white rat, 0.75; guinea pig, 0.45; rabbit (from Luga) 0.5, (from Dorpat) 0.25; white mouse, 0.4; dog (young) 0.23, (full-grown) 0.2; cat, 0.13; fowl, 0.75; duck, 0.25; pigeon, 0.22; bullfinch 0.16. All observers place the frog at over 1.0. In regard to the two most tolerant warm-blooded animals—the rat and the fowl—Clark has shown that no tissue in the rat seems to destroy atropine and other observers have failed to find any destructive power of fowl's serum. As Clark has pointed out, some other reason is needed to explain the tolerance of the rat; and it may also be needed to explain the tolerance of the fowl, though a destructive action of the liver has not been excluded in the latter.

Certainly one of the most remarkable examples of difference of resistance in individuals of the same species is this difference in susceptibility to atropine between rabbits from different districts, and the explanation of this has been shown fairly conclusively to be of the same nature as that of difference in susceptibility between different species. Clark considered that part of this difference might be due to age, as the M.L.D. per kilogram for young rats was about half that for adult rats; but Willberg's experiments in dogs seem to show that in this animal young are more resistant than adults. Perhaps a sufficient number of experiments has not yet been done to determine this age difference which may not be of the same type in all species.

**ACQUIRED TOLERANCE:** *Acquired tolerance to atropine.* Heckel (33) fed guinea pigs, rabbits and rats for long periods exclusively on the

leaves and roots of belladonna or hyoseyamus without producing any ill effects. He claimed that the animals thereby acquired a slightly increased resistance to subcutaneous injection of atropine (M.L.D. 0.6 instead of 0.5), and also to the local mydriatic action. Similar experiments by Lewin (34) gave similar results.

After the discovery by Cloetta, Fleischmann and others that, especially in tolerant animals, the liver and blood possessed the power to destroy atropine, experiments were made to determine whether this power was increased in animals subjected to prolonged dosage with atropine. Cloetta (35) found that in the case of rabbits which had received increasing doses for 6 or 7 months the destructive function of the liver and blood was to an appreciable degree augmented. This did not occur in the cat. In the case of both rabbits and cats the amount and rapidity of excretion in the urine increased in the accustomed animal. He did not determine whether his "immunized" animals could withstand more than the normal M.L.D. Doblin and Fleischmann (28) treated a dog for six weeks with daily injections of atropine and found no atropine-destroying substance, normally present or developed, in the blood. Schinz (29) reinvestigated the problem in rabbits and got varying results according to the degree of natural tolerance in individual rabbits. Like other observers, he found wide individual differences in atropine-tolerance in different rabbits. He confirmed the observation that these differences corresponded with differences in the power of the blood *in vitro* to destroy atropine. He obtained different results from immunization in the two groups. In rabbits with a naturally high resistance, which co-existed with a high power of the blood to destroy atropine, this power *in vivo* and *in vitro* was markedly increased by immunization, whereas in rabbits with a low natural resistance, whose blood had little power to destroy atropine, this power was not similarly increased. He found also that this atropine-destroying function of the blood could be passively conferred upon less tolerant rabbits and cats, by the injection of the serum from a highly tolerant rabbit. Those results are of considerable theoretical importance and require further confirmation. While it would perhaps be unwise to accept them in the meantime without reservation, so far as they go they point to the conclusion that in animals whose blood originally possesses little or no power to destroy atropine this power cannot be originated, still less increased, by immunization, but in those whose blood can, to begin with, definitely destroy atropine this power can be further augmented by immunization.

That the liver, and especially that the serum, of animals which show either congenital or acquired tolerance to atropine should possess the power to neutralize or destroy the alkaloid raises the even more interesting questions as to what is the nature of the substance responsible and also whether it corresponds in any way to the antibodies formed against proteins (toxins). While no final answer to these questions has yet been given, some progress has been made. An estimate of the capacity of the blood to destroy atropine can be obtained from an experiment of Fleischmann who, using a frog heart arrested by muscarine as a biological test for atropine, found that 1 cc. of rabbit's serum could destroy 0.1 mgm. of atropine in 30 minutes. As to the nature of the active substance, Metzner (26) found that the action of serum was destroyed by heating to 60°C. Clark (24) confirmed this and found that the substance passed through a Berkefeld but not a Chamberland filter, acted slowly and in some respects resembled a ferment. Doblin and Fleischmann showed that it did not pass through a Chamberland filter, was resistant to drying, not dialyzable, not a lipid, and due to the albumin fraction of serum. They found that it did not behave toward complement like a true antibody. As to the chemical changes involved in the destruction of atropine, Heffter and Fickewirth (30) found that, of the atropine administered by stomach, part is excreted unchanged in the urine, some as tropine and some as an unknown base. The total amount of bases recovered was equivalent to about half the atropine administered. They found that the rabbit can, to a certain extent, combust both tropine and tropic acid, and they therefore suggested that the disappearance of atropine is due to a splitting followed by oxidation of the components. So far therefore as the present state of knowledge goes it would seem that in cases of congenital or acquired tolerance (individual or specific) to atropine, this tolerance is due mainly to the power of the liver, or of the blood, or of both, to destroy atropine, and this power resides in something of the nature of a ferment or ferments—not yet proved to be the same for both liver and blood—which effect the destruction of atropine by splitting it and then oxidizing its component parts.

In regard to the other alkaloids related to atropine, little work has been done in relation to tolerance. Buys found that the rabbit's liver destroys hyoscyamin, and Doblin and Fleischmann that the rabbit's serum can destroy both hyoscyne and homatropine. A very remarkable fact and one worthy of further investigation has however been discovered by Van Leeuwen (36), namely, that the monkey exhibits an

extraordinary tolerance to hyoscine, as compared with man. This may very possibly be related to the indubitable individual differences in susceptibility to hyoscine observed clinically in man. Blair (37) has pointed out that in cases of addiction to a combination of heroin and hyoscine a considerable tolerance is required not only to heroin but also to hyoscine. It would seem therefore that acquired tolerance to hyoscine is possible in man.

*Acquired tolerance to alcohol.* It is a matter of common observation, or even of experience, that the symptoms produced by alcohol become less manifest with repeated use of it. A very recent statement by Mott (38) may be taken as an estimate of the degree of tolerance which can be successfully attained. "As a result of continued use, tolerance can be acquired so that the habitual drunkard may consume, without becoming intoxicated, quantities of alcohol as beverages which would cause well marked signs of drunkenness, or even prove fatal, to a person not accustomed to it."

Pringsheim (39) was the first to make exact experiments to determine the cause of this acquired tolerance. He produced tolerance in rats and rabbits by administration of daily doses of alcohol for a month. He found that, in rats, equal doses of alcohol produced symptoms of drunkenness conspicuously less marked in the accustomed animal than in the normal animal; and, in rabbits, that a dose of alcohol which produced a deep comatose condition lasting for several hours in a normal animal, produced in the accustomed animal only a moderate stage of excitation. It would seem therefore that, in regard to the acquisition of tolerance to alcoholic intoxication, man can boast of no decided superiority over the lower animals. In explanation of this tolerance his experiments led to the following conclusions. Normal and tolerant animals excrete the same amount of alcohol through the kidneys, lung and skin. The feces in both are alcohol-free. The tolerant animal combusts alcohol quicker—in about two-thirds of the time that is required by the normal animal. The percentage alcohol content of the body in acute alcohol poisoning reaches a higher degree in the normal animal than in the tolerant animal—about 66 per cent more. Tolerance to alcohol is therefore to a considerable degree at least due to quicker oxidation of the poison.

The same type of results was obtained by Schweisheimer (40) in man. He compared abstainers, moderate drinkers and confirmed drunkards. He found that, after equal doses of alcohol, the maximum concentration in the blood was lower, the maintenance of the sustained

high concentration—Grehant's plateau—shorter, and the complete elimination from the blood quicker in the tolerant man than the abstainer.

These experiments seem to prove conclusively that tolerance to alcohol is due partly to increased destruction of it,—according to Pringsheim, mainly by the liver. But, as he has pointed out and as would seem probable from other less exact evidence, the same concentration in the blood produces less effect on the nervous system in the tolerant than in the unaccustomed animal. It is probable therefore that an acquired insusceptibility of the nervous system comes also into play.

Hirsch (43) found that in the presence of oxygen and at 37°C. alcohol is destroyed by the livers of animals which have not acquired tolerance. This destruction was apparently due to a ferment for it was inhibited by heating the liver or by the presence of ferment poisons. The liver paste of rabbits that had acquired tolerance was also active, but not more than that of normal animals. Messner (41) however found no recognizable destruction of alcohol by incubating it for 6 hours at 38°C. with tissues *in vitro*. The last word has not yet been said on the subject for Mellanby (42) states that he has seen no evidence that the rate of destruction is increased in those continually drinking alcohol, nor, if it is taken under constant conditions, of the development of great tolerance.

Pohl (44) was unable to produce tolerance to *methyl alcohol* in dogs, but found that a distinct tolerance, so far at least as the symptoms went, was inducible to *amyl alcohol*. Thus a dog which had been treated with amyl alcohol for over 200 days required at the end of that period 5 cc. to produce the same degree of intoxication as had been produced by 1 cc. in the beginning. It seems probable therefore that the different alcohols vary in regard to the ease with which tolerance can be established to them. Calwell (45) who gave an interesting account of the practice of ether-drinking in Ireland, stated that while the intoxicating dose of *ether* for a novice is from 1 to 4 drams, seasoned toppers can consume large amounts up to 2 or 3 ounces. Also while the beginner has to drink water before and after his dram of ether, the habitué prefers his neat, a distinction which he shares with the Highland ghillie. A local and general tolerance can therefore be acquired to ether probably very similar to alcohol tolerance. The same is true of paraldehyde though the habit is rare.

*Acquired tolerance to artificial hypnotics.* Man will develop a habit and acquire a certain amount of tolerance to nearly all hypnotics. There is perhaps no well-defined tolerance to sulphonal or bromides for, with them, tolerance is complicated by the slowness of their excretion; but with most of the others it is well known that tolerance can be acquired. It is not easy to obtain sufficiently reliable data to estimate the degree to which tolerance can reach, but in regard to the two most important hypnotics, chloral and veronal, it seems unquestionable that men can come to withstand much more than the normal dose, in the case of veronal possibly even lethal doses. Not much work has been done to explain tolerance to these substances.

Wallace (46) gave gradually increasing doses of *chloral* to dogs by stomach, and found that so far as symptoms of depression of the nervous system were concerned, only a slight degree of tolerance was developed, comparable to that displayed to alcohol. Biberfeld (47) investigated tolerance to certain groups of hypnotics. With *amylen hydrate* he could produce no tolerance in rabbits; but in a dog, in which, to begin with, 4 grams of amylen hydrate produced sleep, 8 grams failed to do so after the animal had received repeated injections for 24 days. With *chloral* he found that a dose which produced sleep in a dog ceased to be operative after chloral had been administered regularly for five weeks. He could produce no tolerance to *sulphonal* or to the urea derivatives, urethane, bromural or *veronal*. Bachem (48) gave a dog subcutaneous injections of sodium veronal every three days for about two months but found no evidence of acquired increased destruction as shown by the amount of veronal in the urine. (It is not made clear from his account whether his animals really developed any great tolerance.

Chloral is mostly reduced in the tissues to trichlorethyl alcohol which combines with glycuronic acid to form a physiologically-inert urochloralic acid, in which form it is excreted in the urine. Wallace thought that the formation of this combination might possibly keep pace with the increasing amounts of chloral given and by means of this protective agency a tolerance be established. He came to the conclusion however that this combination could not bring about any great tolerance. Biberfeld came to the same conclusion in regard to amylen hydrate, which also unites with glycuronic acid.

*Acquired tolerance to cannabis indica.* A diminishing susceptibility to the action of *Cannabis indica* probably occurs with habitual use. Marshall (49) found that a definite tolerance could be produced in dogs.

Fraenkel (50) found that rabbits possess a very high congenital tolerance to indian hemp, in fact they seemed to be almost completely refractory, no effect being produced in them by a dose 100 times greater than was needed to produce typical haschisch sleep in dogs. Tolerance was rapidly induced in dogs to cannabinol, but this tolerance seemed to be limited to the action on the nervous system, for the animals lost much weight during the time of immunization. Beyond the fact of its occurrence, nothing is known of the nature of this tolerance.

*Acquired tolerance to opium alkaloids: a. Morphine.* It is familiar knowledge that continued use of opium or morphine in man may lead rapidly to a habit and to a very remarkable degree of tolerance. The M. L. D. of morphine for man by mouth has been estimated at about 3 to 6 grains. McIver and Price (51) found the average daily dose of morphine, in a group of people addicted to its use, to be about 15 grains, and in one case as high as 90 grains. Wholey (52) found that one of his patients had been taking daily for six weeks 25 grains of morphine hypodermically, and another 60 grains by mouth. Though some habitués have made exaggerated statements as to the amount of opium or morphine they consumed, it is beyond dispute that, by continued use, man may come to withstand, without fatal results, many times the dose that would be lethal for one not accustomed to the drug. Habit and tolerance are produced by absorption through any channel by which the drug can gain entrance to the body, e.g., by opium smoking or ingestion, by hypodermic injection of morphine, or by snuffing of heroin. Habit and tolerance are induced not only to morphine but at least to those of the opium alkaloids which are closely related to it. Wholey (52) found one of his patients taking daily 17 grains of heroin, though the lethal dose of heroin is less than that of morphine. It seems indubitable that codeine is less likely to form a habit, though Pelz (53) has recorded a case of a man who developed a habit for codeine and who eventually took 25 grains a day. An unfortunate sequel to habit is the occurrence of disagreeable and even dangerous symptoms ("abstinence" symptoms) if the habit is suddenly broken off. No experiments have to my knowledge been made in man to determine the cause of this acquired tolerance, but a very large number has been made on laboratory animals, in certain of whom at all events, especially the dog, a high degree of tolerance can be produced. Only the most important of these investigations will be considered.

Exact experiments instituted to determine the nature of morphine tolerance were possible only when something decisive became known

concerning the fate of morphine in the body. Tauber (54), who gave an excellent account of the previous literature on the subject, first enunciated that in the dog the main channel of excretion of morphine, when injected hypodermically, is by way of the alimentary tract, only negligible quantities being eliminated in the urine. He was able to recover in the feces over 40 per cent of the morphine hypodermically administered. Accepting and confirming Tauber's view that only negligible quantities of morphine were excreted in the urine, Faust (55) proceeded to investigate the changes that occurred in the elimination of morphine in the feces of dogs who had been given gradually increasing doses of the alkaloid and who had developed a well-marked tolerance to its actions. The results he obtained were remarkable. He found that, whereas in acute morphine poisoning about three-fifths of the morphine could be recovered in the feces, the amount found there, when increasing doses were given daily for weeks and tolerance gradually established, rapidly and regularly dwindled until in less than two months no trace of morphine could be discovered in the feces. When the animal was killed at this point no morphine could be found in the liver, spleen, kidneys, or brain. He therefore came to the conclusion that the tolerance which is acquired to morphine is due, not to an accustoming of the tissues themselves, not to a lessened susceptibility on their part to its action, but to the development on the part of the organism of the ability to destroy increasing quantities of morphine.

Cloetta (56) realized that Faust had not revealed by what way or in what organs morphine was destroyed, and also pointed out a serious objection to accepting increased destruction as the sole explanation of morphine tolerance, namely, that this destruction would have to proceed with an almost incredible celerity to explain the complete absence of symptoms in an immunized animal injected subcutaneously with a lethal dose of a rapidly absorbed substance like morphine. He therefore sought a supplementary explanation. He confirmed the observation that the dog excretes large amounts of morphine in the feces, though he found less (23 to 32 per cent) than was found by Faust. In regard to the fate of morphine he found that, injected intravenously, in dogs and rabbits, it disappeared almost completely within five minutes, and completely within twenty minutes, from the blood, a result similar to that previously obtained by several observers. From experiments *in vitro* he concluded that morphine is not destroyed by blood and must therefore be destroyed by other tissues. When an animal (dog or rabbit) was killed after an injection of morphine and at a time (12 to

60 minutes) when its action was marked, no morphine could be found in the brain and little in the liver. Emulsions of brain or liver to which morphine was added were centrifuged and larger quantities of morphine were found in the brain sediment than the liver sediment. He concluded that brain tissue had a greater affinity for morphine than liver tissue. From the fact that brain incubated with morphine *in vitro* destroyed more morphine than liver tissue, he argued that the reason no morphine was found in the brain after injection—in spite of the fact that brain had a greater affinity than other tissues for morphine—was due to the fact that brain destroyed the alkaloid. He proved that it was actually destroyed by the fact that in rabbits and pigeons killed by injection of morphine, less than two-thirds of the amount injected could be recovered in the whole carcass. He found that in a tolerant rat or pigeon (daily injections for 7 or 8 months) morphine disappeared from the body within two days, but not within four hours, after the last injection. The rate of destruction did not appear to be much quicker than in the normal animal though from a comparison of the destructive actions *in vitro* of tissues of normal and tolerant animals he claimed to find that in the former the brain had acquired an enhanced power to absorb and destroy morphine. Though Cloetta admitted that his experiments did not solve the whole problem of morphine tolerance, they led to an amplification and revision of Faust's view. Briefly, they claimed to show that morphine is actually destroyed, not hidden, in the body; that this destruction is effected largely by the brain itself; but that even in an immunized animal the destruction though greater than in a normal animal is not sufficiently complete altogether to explain the tolerance.

Modification of Faust's theory was further necessitated by experiments of Rubsamen (57). He compared normal and immunized rats in regard to their power to destroy morphine as estimated by the amount recoverable from the whole carcass, and found that, coinciding with the production of tolerance, there occurred an ability to destroy larger quantities of morphine more completely and more rapidly. So far his experiments were in agreement with those of Faust and Cloetta. But this destruction did not occur sufficiently quickly to explain the tolerance, because rats which could withstand 2 M. L. D. with impunity were found to contain in their tissues an amount of morphine sufficient to produce profound toxic effects in unimmunized animals. In regard to the site of this destruction, using an improved technique, he failed to find any destruction *in vitro* by the brain of the

dog, rabbit or rat, either normal or immunized, being thus in complete contradistinction to Cloetta. He concluded that there were two causes of morphine tolerance, in the first place an increased power of destroying morphine (not explained by *in vitro* experiments), and secondly, a diminished susceptibility of the tissues to its action—an assumption necessary to explain the fact that, for some time after injection, a quantity of morphine could be found in the immunized animal (showing no symptoms) which would produce profound effects in an unimmunized animal.

Albanese (58) found that the liver of a normal dog had practically no power to destroy morphine, nor had that of an immunized dog (daily injections for 3 months) if the liver were taken a few hours after the last injection; if however the immunized dog were killed 60 hours after the last injection, then the liver possessed an extraordinary power to destroy morphine. Dorencourt (59) contradicted these results in their entirety, for he found that the liver of both normal and tolerant dogs destroyed morphine (the latter to a greater degree and in proportion to the tolerance developed), but that there was no increase during the abstinence period such as was described by Albanese.

It is difficult to come to a conclusion from results so divergent. Even if these *in vitro* experiments on morphine destruction were in agreement, they would still have to be reconciled with older experiments of Tauber (54) who found that when blood containing morphine was perfused through the liver and kidney of the pig, practically none was destroyed (other than could be accounted for by unavoidable loss by the method used) though the morphine-containing blood was reperfused through these organs for about a score of times for a period of about two and a half hours.

As if confusion were not already sufficiently confounded, Kauffmann-Asser (60) obtained results which in points of essential importance disagreed with those of his predecessors from Tauber onwards. After a single injection of morphine in rabbits or dogs he found amounts varying from 5 to 30 per cent of the morphine in the urine. In a dog which received daily injections for 17 days, 3 to 13 per cent was recoverable in the urine, and the amount did not fall off in that time. A remarkable result was obtained in the rabbit immunized by daily injections, for the amount of morphine in the urine increased from 5 per cent to begin with to a maximum of 39 per cent on the 12th day, and then fell gradually to nil on the 22nd day. While therefore other observers had proceeded on the fundamental assumption that the

excretion of morphine in the urine is negligible, Kauffmann-Asser found amounts in the urine sometimes even greater than in the feces. These experiments can be disregarded if the recent criticism of Oshika (61) is true, namely, that the method used by Kaufmann-Asser is not applicable to the estimation of morphine in the urine.

These experiments have attempted to solve the problem of morphine tolerance by a quantitative comparison of the amounts destroyed in a normal and tolerant animal respectively, but the problem has been attacked from other angles. Van Egmond (62) found that, in dogs, morphine in doses of 0.04 mgm. per kilo upwards produced a marked slowing of the pulse which he experimentally located to a stimulation of the vagus center. When a dog was immunized by increasing doses until at the end of about three months it was receiving 0.23 mgm. per kilo, it was found that this dose no longer produced the original symptoms of vomiting, narcosis, etc., but still at this time a dose of 1 mgm. per kilo lowered the pulse rate from 120 to 82 for five and a half hours, just as it had done before immunization. In other words, though no tolerance had been gained so far as the vagus center for the heart was concerned, a tolerance had been gained against the symptoms of narcosis, vomiting, etc., to a dose 230 times greater. He drew the only obvious conclusion from this, namely, that tolerance could not be due to increased destruction of morphine, for in that case it would be impossible to explain the fact that in the tolerant animal a minimal dose still produced the same effect on the vagus center for hours after injection as it had originally produced in the unimmunized animal. Increased destruction of morphine at all events of the type adumbrated by Faust and others could not explain a tolerance which was limited to certain tissues only. Essentially the same phenomenon was shown in a slightly different way by van Dongen (63) who found that in a dog that had received, in two months, doses increasing from 10 to 200 mgm. of morphine, while the respiratory center had become tolerant of 1800 times the smallest active dose, the vagus center for the heart was, as van Egmond had found, still as sensitive to the minimal active dose as in the normal dog. He further arranged the descending order in which the centers of the dog acquire tolerance as follows:—pupil, vomiting, diarrhea, narcosis, respiratory center, and lastly the vagus center which last indeed seemed to acquire no tolerance at all. Especially as this order did not correspond with the order of susceptibility of the different centers in the normal animal, he came to the same conclusion as van Egmond that, whether or not an increased

destruction also takes place, the development of a tissue immunity must also be assumed to explain morphine tolerance. He obtained no comparable results in rabbits, indeed he obtained no evidence of tolerance of any kind in this animal by injections going on for six weeks. Tamura (64) has also found that the vagus center still retains its sensitiveness to morphine in tolerant animals.

Other views on morphine tolerance can be dismissed more briefly. Hirschlaff (65) claimed that the serum of rabbits alleged to be immunized to morphine contained an anti-morphine and that this serum if previously injected into control animals would protect them from lethal doses of morphine. But the numerous experimental errors upon which this conclusion was based were promptly pointed out by Morgenroth (66); and Hirschlaff's results have been contradicted by every subsequent observer, up to, more recently, Pellini and Greenfield (67). Marmé's (68) oxidimorphine theory has also been discredited, though Valenti (69) has revived it to the extent that he claims that the serum of abstinent dogs can produce circulatory disturbances in normal dogs. He has not found what in the serum produces these effects, and his experiments require confirmation.

*b. Other alkaloids of the morphine group.* Habit and tolerance are common in man to heroin, rare to codeine. Bouma (70) found that in dogs 80 to 90 per cent of codeine injected subcutaneously could be recovered in the urine and feces, the urine containing more than ten times as much as the feces. After prolonged administration no tolerance was produced but rather an increased susceptibility; the organism did not acquire the power to destroy more codeine. Biberfeld (71) found that dogs by repeated administration soon became insusceptible to the sedative action of eucodal or of paracodeine but not to the stimulant actions. Babel (72) found that the brain tissue of rabbits immunized to morphine could destroy heroin but not codeine, and that a pigeon immunized up to 3 M.L.D. of morphine was still normally susceptible to codeine and unable to destroy the latter alkaloid in its tissues. Langer (73) found that in the normal dog heroin was mostly excreted unchanged in the urine, a smaller amount appearing in the feces as an undetermined base, but in a dog immunized by injections for two months no heroin could be found in either urine or feces. He found that tolerance was rapidly produced to the depressant action of heroin but not to the convulsive action. He was unable to produce tolerance to heroin in rabbits.

To sum up the question of acquired tolerance to these morphine alkaloids, it may be said that tolerance is acquired easily to morphine, less easily to heroin, and with great difficulty to codeine. It is most easily produced in man, less easily in dogs, with difficulty, if at all, in rabbits, and not at all—according to Hausmann (74)—in frogs. Tolerance is most easily acquired to the depressant action on the nervous system, far less to other actions. It is chiefly due to an acquired insusceptibility of the nerve cells to this depressant action. Most observers (Faust, Cloetta, Rubsamen, Albanese with morphine and Langer with heroin) agree that there is also acquired during tolerance an increased power of destroying morphine, but as to the details of this process there is no convincing agreement.

*Crossed tolerance to hypnotics.* Clinical observation seems to have established beyond controversy the fact that people who, by long and faithful use, have acquired an increased tolerance to alcohol, become thereby less susceptible not only to such anesthetics and hypnotics as ether and paraldehyde but also to chloroform, chloral and even veronal. There is also a belief, less well founded, that a person who has acquired tolerance to one cerebral depressant will tolerate larger quantities of other, even chemically unrelated, narcotics. The question of crossed tolerance must obviously throw light on the nature of the original tolerance, and the experiments bearing on it may be discussed here together.

There would be no inherent improbability in supposing that a mechanism which the organism could develop for destroying alcohol more completely, would also effect an increased destruction of ether. Ether however is not destroyed in the body but excreted unchanged. Ether tolerance in alcoholics cannot therefore be due to increased destruction of ether, but rather to an acquired insusceptibility of the nervous system. The presumption is that the same thing occurs with alcohol itself. Apart from the still greater unlikelihood that a destructive mechanism would apply both to alcohol and chloroform, it could hardly operate with sufficient rapidity to interfere with the quick onset of anesthesia produced by chloroform. There is therefore a twofold reason for ascribing chloroform tolerance in alcoholics to an acquired insusceptibility of the nerve cells rather than to an enhanced destruction of chloroform. As it is almost incredible that such a diminished susceptibility should not apply to the substance to which tolerance was originally established, this, apart from other evidence goes far to prove that acquired tolerance to alcohol is due to acquired insusceptibility

of the nerve cells, and that this conveys an insusceptibility to other cerebral depressants.

In regard to other narcotics experiments have been made chiefly by Myers (72) and Biberfeld (75). Myers determined the effects of various hypnotics upon normal dogs as compared with dogs previously rendered tolerant to morphine. He found that in the latter crossed tolerance existed to heroin and codeine. This was manifested chiefly as regards their actions on the respiration and equilibrium, but not on peristalsis. No crossed tolerance was found to cannabis indica or chloral. He concluded that a cross tolerance may exist between closely related drugs but that this is evidenced only on those functions on which the drugs have a common selective action. Biberfeld found, on the other hand, that morphine-tolerant dogs did not acquire tolerance to hyoscine, cocaine, or even to heroin. Though these observations are not sufficiently numerous to enable any comprehensive generalization to be drawn from them, it seems more than probable that acquired tolerance to one narcotic confers some tolerance to other narcotics and that this tolerance is more or less limited to actions which they have in common.

*General conclusions as to acquired tolerance to narcotics.* The foregoing account of the present state of knowledge in regard to acquired tolerance to hypnotics would have little value beyond a mere catalogue of results, were no more general conclusions deducible from them. A wide survey seems however to reveal certain points of unanimity that may now be summarized, even if they be regarded in the meantime as merely provocative. (1) Among drugs generally, it is chiefly to depressants of the central nervous system that tolerance can be acquired,—to alcohol, chloral, morphine, etc., rather than to, e.g., codeine, strychnine or other alkaloids. (2) When a substance combines a depressant action on certain parts of the nervous system with a stimulant action on other parts of the nervous system or with other physiological actions, it is to the former chiefly or only that tolerance is established. The experiments of Van Egmond, Van Dongen and Tamura with morphine, of Langer with heroin, and of Biberfeld with paracodeine are all in agreement on this point. (3) In regard to these hypnotics therefore acquired tolerance is largely if not entirely due to the fact that certain parts of the central nervous system can acquire an increased resistance to the action of depressants. (4) It may not be accidental to this that tolerance can be acquired the more easily in different animals in proportion to the higher development of the brain, e.g., in the order

man, dog, rabbit, frog. (5) The fact that some degree of tolerance can be acquired to alcohol, chloral, veronal, morphine, cannabis indica, hyoscine, substances with little or no chemical similarity but which agree in being cerebral depressants, suggests strongly that tolerance is more intimately connected with their actions on the nervous system, in which they agree, than with increased destruction of them, in the mechanism of which they must necessarily disagree. (6) Experiments dealing with destruction of alkaloids, etc., by the tissues are not only negatively useless but may be positively misleading unless a very exact method of quantitative estimation is employed.

*Acquired tolerance to cocaine.* That habit and tolerance to cocaine can be developed in man is only too well known. Tolerance can be acquired very rapidly (76); for example, the daily dose may be increased from 0.1 gram to 3 grams in two months. Grode (81) summarized the literature previous to 1912 dealing with cocaine tolerance. From various published accounts he estimated the M. L. D. for man by hypodermic injection to be from 0.22 to 1 gram; whereas the daily dose taken subcutaneously by an habitu   might be as great as 8 grams. There seems no reason to doubt, as some have done, that man can acquire by habituation a high degree of tolerance to cocaine. Little or nothing is yet known of the cause of this tolerance partly because of experimental difficulties arising from the fact that nothing like the same degree of tolerance, if any, has so far been produced in laboratory animals.

Out of a large number of experiments on mice, Ehrlich (77) obtained only a slight and doubtful tolerance in a few animals. Wiechowski (78) could not produce tolerance in dogs (17 injections) nor Von Anrep (79) in rabbits. Ritter (80) after repeated intravenous injections in dogs claimed to observe a diminished intensity of action, but he does no more than mention the fact. Grode (81) could not obtain any evidence of acquired tolerance to cocaine in guinea pigs, rats or dogs, but rather an increasing susceptibility. Mills (82) obtained no cumulative effects in rabbits from repeated injections provided that the intervals between the injections were more than 75 minutes, this being taken to indicate rapid destruction or elimination of cocaine. Rifatwachdani (83) found that the excretion of cocaine in the urine increased by "prolonged" injections but he gave cocaine only for 9 days and his experiments merely show that the quantity of cocaine he gave was not excreted within 24 hours and therefore with daily injections the amount increased daily. He found that when cocaine was left for several hours in a ligatured limb of a living animal, most of it could be recovered,

from which he deduced that cocaine is not to any great extent destroyed by living tissues, but his methods were not sufficiently quantitative to decide this. Wiechowski (78) also found that, of cocaine shaken with liver or muscle juice for 5 hours in vitro, 80 per cent was recoverable. Eggleston and Hatcher (84) on the other hand perfused a cat's liver with defibrinated blood containing cocaine for one hour at 37°C. and calculated, from a biological test, that not less than half had been destroyed.

No conclusions can be drawn as to the nature of acquired tolerance to cocaine from these meager results. If it be true that tolerance to this alkaloid can be obtained only in man, then naturally the solution of the problem can be looked for only from experiments on man himself.

*Acquired tolerance to caffeine.* As a result of the colossal experiment that has been made, it would be perhaps the general judgment that, in spite of years of indulgence in caffeine-containing beverages, man acquires only a slight degree of tolerance to their effects. No such increase of dose is needed of caffeine as of cocaine, though these alkaloids resemble one another to some extent in their actions on the central nervous system. Experiments on laboratory animals point to a similar conclusion. Boek and Larsen (85) found that, so far as the effects of toxic doses were concerned, only a very limited tolerance to caffeine could be produced in rabbits by repeated injections. This slight tolerance was due not to increased excretion of caffeine but probably to increased destruction of it and to lessened susceptibility of the tissues to its action.

Myers (86) gave rabbits daily hypodermic injections of 100 mgm. of uncombined caffeine for a period of several months and found that, to produce minimal diuresis, twice as much caffeine was needed in these habituated animals as in normal animals. A definite tolerance had therefore been gained to the diuretic action of caffeine. As this was determined by intravenous injection, it pointed to the tolerance being due to an acquired insusceptibility of the kidney cells, though an increased destruction could not positively be excluded.

*Acquired tolerance to nicotine.* It is beyond question that the majority of men can acquire a certain degree of tolerance to tobacco smoking, and experiments to find out whether a similar tolerance can be induced in laboratory animals to nicotine, and to determine the cause of this tolerance when induced, have frequently been made. As a summary of most of the existing work on nicotine tolerance has already been given (91), and as the results obtained by different in-

investigators are in most points in agreement, it will be possible to indicate at no great length the present state of knowledge of the subject.

Esser (87), employing large doses, found that in dogs and rabbits a well-defined tolerance to nicotine could be obtained whether the alkaloid were given subcutaneously or by mouth. Hatcher (88) found in rabbits that tolerance was more readily induced by large doses at intervals of 3 days than by more frequent repetition of smaller doses. In any case the tolerance was limited though, once gained, it was retained for some time. Adler and Hensel (89) did not obtain evidence of acquired tolerance in rabbits by intravenous injection.

Edmunds (90), giving nicotine chloride by stomach-tube to cats, found that they became more susceptible (daily doses for a month). He found however that dogs which received subcutaneous injections about every second day, though they showed a similar increased susceptibility for the first six weeks, later began to gain a slight tolerance so that at the end of another three weeks they were able to take twice the original emetic dose without vomiting. With massive doses, after Esser's method, tolerance was apparently quite easily gained. Dixon and Lee (91) found that the liver, and to a less extent the brain and muscle of a tolerant rabbit destroyed more nicotine than the corresponding tissues of a normal rabbit. The substance responsible for the destruction of nicotine was not dependent upon the presence of living intact cells, but in regard to resistance to heat drying, etc., behaved like a ferment. "These experiments show that a certain small degree of tolerance can be obtained to nicotine, and that this is brought by the destruction of the alkaloid. The destruction goes on very slowly, and it can never be accelerated to such a degree that an injection of a poisonous dose of nicotine into the circulation of an animal will lose any large amount of its effect. If the nicotine reaches the circulation slowly and in minute quantities it may be dealt with by the tissues, and this is the condition which we may assume obtains during tobacco smoking."

Edmunds and Smith (92), in replying to certain criticisms which Dixon and Lee had pronounced upon Edmunds' previous experiments, showed that the livers of all dogs possess some power of destroying nicotine but that there were just as great differences in different normal animals as these authors had found between normal and tolerant animals. They therefore did not consider it proved that tolerance was accompanied by any increased power of the liver to destroy nicotine. Clark (93) found that the frog's liver did not destroy nicotine.

So far, then, as nicotine is concerned, experiments are concordant in that they show that tolerance to this alkaloid can be induced in animals by repeated administration—less readily by small than by large doses. The degree of tolerance attainable either in lower animals or in man does not seem to be nearly so high as that induced to morphine. The determination of the cause of the tolerance is therefore more difficult in proportion to the slightness of the tolerance. As to whether the tissues of a tolerant animal destroy more nicotine than the corresponding tissues of an unimmunized animal, the same divergency of results has been obtained as with other alkaloids. That, in the case of nicotine, tolerance is accompanied by increased destruction can hardly be taken yet to be established. No one has made any attempt to discover what becomes of the nicotine when destroyed, e.g., by the liver, though if, by the employment of larger amounts of material, the stages of disintegration could be revealed the results would be interesting.

No work has been done on congenital tolerance to nicotine. The M. L. D. per kilo for the rabbit is stated to be 10 times that for the dog. If congenital tolerance is also due to superior power in the more tolerant animal to destroy nicotine the possibility is promising that the tissues of the rabbit would show a superiority of destructive power over those of the dog—a difference beyond experimental errors in proportion as the difference in tolerance is wide. Hatcher made the interesting observation that young guinea pigs are more susceptible to nicotine than adults, the M. L. D. for the former being 0.15 mgm. per kilo as compared with 0.4 for adults. The explanation of this age difference is not yet forthcoming.

*Acquired tolerance to arsenic.* It is not necessary here to revive the old dispute about the authenticity of arsenic-eating, for it is now accepted that man can, by habituation, come to tolerate with impunity doses of arsenic which would produce serious effects in people not accustomed to it. Nor need the older literature on the subject be traversed, as summaries of it are already accessible, e.g., by Maclagan (94) and Hausmann (95). The interest which attaches to arsenic tolerance is due largely to the fact that, while acquired tolerance to organic substances is not uncommon, arsenic is almost singular among mineral substances in this respect. Possibly too, the subject has acquired added interest of recent years from the discovery that many protozoa can acquire a high tolerance to arsenic, though there may be little in common between this tolerance and that which is acquired by mammalia to arsenic.

Apart from other difficulties, such as that of accurate quantitative estimation of arsenic, there are two complications which have impeded the study of arsenic tolerance. One is that there are wide individual differences in resistance to arsenic. This has not only been alleged on good grounds for man but has clearly been shown in laboratory animals. The other—a very familiar difficulty—is that the statements in regard to the toxicity of arsenic are so conflicting. Hausmann in his review (95) estimates that the M. L. D. for man is somewhere about 0.1 to 0.2 gram, and this estimate is commonly accepted in textbooks. On the other hand Bradshaw (96), following an older practice, used to give in malaria as much as 120 minims of Fowler's solution (= 0.072 gram arsenious acid) in single doses for three days in succession and "never met with an instance of consequent stomach or any other inconvenience." This was 15 times the maximum usual therapeutic dose and was in fact not far short of a lethal dose according to the usual estimate, and the arsenic in Fowler's solution is in a soluble form.

Cloetta (97) gave increasing doses of arsenic to rabbits and dogs and succeeded in producing definite tolerance, especially to dry arsenious acid. One dog treated for over a year finally received a dose of over 0.4 gram per kilo. He found *a*, that the amount of arsenic in the urine did not rise in proportion to the increase of dose but remained constant, the percentage falling enormously; and *b*, that a dog tolerant of 2.5 grams by mouth was killed by a single hypodermic dose of 40 mgm. He concluded from these facts that tolerance to arsenic was not accompanied by any general tolerance but was due to a diminished permeability of the mucous membrane of the alimentary canal to arsenic. There were two defects in his experiments—first, that only a few irregular estimations of arsenic in the urine were made and it is known that the amount may vary considerably from day to day; and second, that the oft-quoted experiment on the difference in tolerance to stomach and subcutaneous dose was hardly decisive, for the dog received 2.5 grams by mouth one day and 40 mgm. hypodermically the following morning, whereas there ought to have been an interval of a day or more between the two doses. However the contention that there is developed a local tolerance of the mucous membrane of the alimentary canal to dry arsenic has been confirmed in several ways and may be taken to be established.

Hausmann (98), by administering for over a year repeated doses of dry arsenious acid by mouth, immunized a dog to tolerate 0.06 gram per kilo. He found that while the percentage excretion in the

feces diminished from 77 to 30, the percentage excretion in the urine remained stationary at about 5. He concluded that in the course of the development of tolerance the method of excretion was altered and that arsenic was excreted in a combined form not recognizable by ordinary methods.

The question has recently been reinvestigated by Joachimoglu (99). He immunized two dogs (which showed marked differences in resistance) one to 0.056 gram per kilo in 6 months, and the other to 0.025 gram per kilo in 13 months. Arsenious acid was given dry by mouth. He differed *a*, from Cloetta in finding that the absolute amount of arsenic in the urine increased fourfold though the percentage diminished from 15 to 6; and *b*, from Hausmann in finding that not only the absolute but the percentage amount increased in the feces. His experiments therefore showed that with increasing doses the absolute amount of arsenic absorbed and in the circulation increased, and therefore tolerance could not be due entirely to diminished absorption. He substantiated the fact that a local tolerance was developed by microscopic examination of the intestine, the mucous membrane of which in the tolerant dog showed none of the changes which would have occurred after much smaller doses in that of an unimmunized dog. Also a normal dog killed by a large dose of arsenic was found to absorb 31 per cent of the dose given, 8 times as much as he calculated a tolerant dog would have absorbed of the same dose, proving that absorption is diminished in the tolerant animal.

The position now seems to be therefore that tolerance to arsenic can be induced in man or animals when arsenic is given by mouth undissolved, it can be induced much less readily if arsenic is given by mouth in solution, and has not so far been produced by subcutaneous injection. This tolerance is partly due to an acquired insusceptibility of the mucous membrane of the alimentary canal, as the result of which larger doses are required to produce vomiting and diarrhea. About this there seems to be no doubt. Tolerance is also due in part to a diminished absorption of arsenic; the mucous membrane ceases to absorb arsenic in proportion to the increased amount in the lumen, and this effect is probably related to the acquired insusceptibility of the mucous membrane to arsenic. Owing to this protective mechanism there is not a rise in the concentration of arsenic in the blood proportionate to the increase in dose. It seems almost conclusively proved that this diminished absorption goes on *pari passu* with increasing tolerance, when arsenic is given dry by mouth.

Two points of doubt remain. In the first place it is not certain whether any cellular tolerance is gained by tissues other than the alimentary mucous membrane. According to Joachimoglu, when large doses are given to a tolerant animal the actual concentration in the blood may be high, though not of course in proportion to the dose. As to whether in these circumstances a concentration in the blood is reached which, though innocuous to the tolerant animal, would be injurious to an unimmunized animal is not yet decided. The second difficulty is that no satisfactory reason has yet been given of why repeated increasing doses should in some cases produce tolerance, while in other cases, doses, smaller and given for a shorter time, will produce chronic (? cumulative) poisoning. In the absence of adequate data, speculation on this point would be idle.

*Destruction of drugs by the body tissues.* One very important problem which has repeatedly arisen in the foregoing discussions relates to the methods by which drugs are destroyed in the body,—whether, for example, congenital tolerance is due to superior destruction of the drug by the more tolerant animal, and whether destruction can be increased by habituation. The experimental investigation of these problems is difficult because it is not an easy task to follow the fate of a drug quantitatively from the time of its injection to the time of its complete disappearance from the body, and this difficulty is reflected in the conflicting results which have been obtained. It is believed that alcohol, atropine, morphine, strychnine (100)—to mention only a few drugs—are partly destroyed in the body, that this destruction is due to ferment action, and that the liver is the organ usually chiefly responsible for the destruction. There is no difficulty in imagining that alcohol may be destroyed in the body, for according to some it is normally present in the blood in small quantities, and in any case is closely related to substances which the body normally destroys. On the other hand it can hardly be supposed that there are *specific* ferments in the body which are lying in wait in the hope that some day a suitable alkaloid will be presented to them for attack. It is highly probable therefore that if alkaloids are destroyed by ferments, the usual business of those ferments is to destroy substances normally occurring in the body, e.g., decomposition products of proteins, amines, etc. It may well be that different animals, according to differences in their diets, etc., may have different powers of destroying alkaloids, and that congenital tolerance may be due sometimes to these differences.

When it comes however to the questions *a*, whether an animal can acquire by habituation a destructive action which it originally did not possess; or *b*, whether it can by habituation increase such a power if preëxistent, the difficulties are greater. In regard to the first question, the destruction of the benzene ring compounds may afford a parallel. The body can destroy the benzene ring of those aromatic aminoacids which enter into the composition of proteins, whereas the benzene ring of foreign aromatic compounds is usually left intact. The condition of alkaptonuria especially shows that when there is a congenital absence of the enzyme which destroys homogentisic acid, this ferment action is never gained throughout life, though the substance to be destroyed is being constantly formed from the food. This seems to be one certain example of the complete inability of the body to form a new enzyme in response to habituation (101).

In regard to the second question, it is quite possible that ferment activity can be artificially increased, because animals fed for a long period on one particular kind of food may develop an abnormal ferment activity for this particular food. The difficulty in regard to, e.g., alkaloids would seem to be this. The ferment that destroys them is one that presumably normally destroys some other substance and it is difficult to imagine how this ferment can be increased by the minute quantities of alkaloid (e.g., of nicotine) which enter the circulation. In other words, one would imagine that there would be even larger fluctuations from day to day in the amount of substance that the ferment normally destroys than would be afforded by the addition of the alkaloid.

At all events these considerations would seem to urge a caution against accepting results which have claimed to show an augmentation of destruction of alkaloids given in small amounts and for short periods and especially those results which claim that a new power of destruction can be originated by habituation.

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*Destruction of Drugs by the Body Tissues*

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## DESTRUCTION OF THE RED BLOOD CORPUSCLES IN HEALTH AND DISEASE

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The subject of blood destruction has almost as many ramifications as the blood stream itself; and in entering upon it one must choose a path or perhaps be lost amid data. In the present instance an effort will be made to follow out the physiological processes involved in the fate of the cells, with incidental digressions only for the multifarious agents causing cell injury, and the innumerable consequences thereof.

THE EVIDENCE FOR A NORMAL DESTRUCTION OF BLOOD. So subtly is normal blood destruction conducted and the remains of the cells disposed of that were it not for indirect evidence one might suppose the life of most red corpuscles to endure with that of the body. But this evidence is convincing. The continuous activity of a broadly distributed hematopoietic tissue; the daily excretion through the bile of a pigment nearly if not precisely identical with one of the pigmented derivatives of hemoglobin (220), (221); the appearance of this derivative in old hematomas (224) and in the plasma after hemoglobin injection and liver exclusion (236); the apparently significant association of hemoglobin and bilirubin throughout the animal kingdom (143); the existence here and there in the healthy organism of cells containing erythrocytes in various stages of disintegration; and, not least, the delicate structure of the red cell itself, its lack of a nucleus, the incessant squeezing and buffeting to which it is subjected,—these and other facts clearly prove that blood destruction must be one of the routine tasks of the body. Yet this statement of the case would be one-sided without a mention of the corpuscle's notable aptitude for the existence that it has to lead. Chemical wear and tear are practically negligible in it, as shown by the fact that it uses up almost no oxygen (133), (134), (82); it is almost devoid of autolytic ferments (159), (188), (189), (162); the corpuscular tissue is so constituted as to withstand sudden and considerable changes in the tonicity of the surrounding medium, such as the cell may not infrequently have to encounter; and while yielding is yet so resilient that the disc shape is retained although the cell is forced over and over

again through channels having but a fraction of its diameter. The fluid, too, in which the corpuscles are carried not only acts to protect them against mechanical insult (175), but has other and diverse "buffer" potentialities (235).

**THE RATE OF NORMAL BLOOD DESTRUCTION.** Were the rate of blood destruction known, one might be better able to judge the adequacy of the physiological mechanisms which have been invoked to accomplish it. But until recently practicable methods have been lacking even for the measurement of the amount of blood possessed by the body at any one time. The development of means to determine plasma volume (99), (129) and total hemoglobin (77), (79) has filled this gap. Yet so aided one ascertains with much exacting labor merely the content of the blood cistern at a special moment. The rates of inflow and outgo, of depletion and renewal, of this content are not touched upon. In the healthy individual these rates must keep pace with each other else the amount of blood would fluctuate more than is the case. It follows that measuring either will give both. All quantitative efforts have centered upon the rate of outgo, that is to say, upon the rate of blood destruction.

The demonstration that hemoglobin injections lead to an increased bilirubin output was a first step in the matter (211). Stadelmann, (206), bringing corroboration, observed that less bilirubin appeared in the bile than was supposedly derivable from the injected pigment and he believed that a part of the latter was utilized in the formation of new red cells. His experiments were only roughly quantitative. That their significance has been overlooked is attested by the many calculations on the normal rate of blood destruction since made which involve the assumption that the bilirubin output furnishes a precise index to the amount of hemoglobin yielded by the destroyed cells. According to one set of figures (76), slightly more than 2 per cent of the body content of corpuscles undergo destruction in every 24 hours; while another (245) has it that five times as much, or about one-tenth the total quantity, is demolished in this time. Recently Brugsch and his collaborators, (37), (38), (39), who almost alone have resorted to experiment in the matter, have followed the bilirubin output in two dogs with bile fistulas, kept suspended in slings, that is to say under highly abnormal conditions, and injected with hemoglobin and hematin. They state that, given a certain formula for hematin, the amount of the substance supposedly yielded by hemoglobin is excreted almost (*fast*) quantitatively as bilirubin; and, with a due recognition of the likelihood of error they go on to figure from the bilirubin output the rate of blood destruc-

tion. Eppinger and Charnass (62) point out that the normal bilirubin yield of fistula dogs is less than half that assumed by Brugsch. On the amended basis they compute that only one thirty-fourth of the blood is daily destroyed. But later authors have in general ignored the correction. And at present the belief,—traceable to the figures of Brugsch and Retzlaff,—is widespread that one-fifteenth of the total blood undergoes destruction each day (1), whatever the animal species.

Experiments lately recorded have been taken to indicate that the quantity of bile pigment manufactured can be greatly increased by carbohydrate administration (237), (239), (240). The protocols leave no doubt that the output of bilirubin was thus rendered more considerable during the period of bile collection. But the said period amounted to only 6 or, at most, 8 hours out of each 24, so the results may have been due to a temporary change in the rate of evacuation of bile pigment from the body rather than to one in the total yield. A like difficulty of interpretation affects observations by the same methods on the bilirubin output following hemoglobin injections (238).

Ashby (6), (7), (8), (9) has procured evidence on the rate of normal blood destruction by following the survival of transfused cells. The blood of individual bulls can be identified with the aid of specifically exhausted isohemolytic sera, and by such means a previous worker had determined the period of survival of cells transfused from one animal (215), (216), to another. The introduced cells were treated from the outset as foreign bodies, and all were removed from the circulation within the extremely short period of 4 to 7 days. But in human beings transfused with blood of a differing but compatible "group," the strange cells would appear to survive for a surprisingly long time. For example, in 10 cases requiring transfusion because of secondary anemia, 40 to 50 per cent of them were still to be found after 28 to 52 days. In a healthy man some persisted for 100 days. There is a periodicity in their elimination. In women they are removed during the menses more especially.

How is one to reconcile such longevity of the cells with the data yielded by the bilirubin output? It has been suggested by some observers (231), who confirm Ashby's transfusion findings, that the alien cells may so far differ from those proper to the host as to be less susceptible to the ordinary processes of destruction. And it is not inconceivable that the stroma constituent of the strange corpuscles whereby they become recognizable does not disappear with the cells but, incorporated in new ones put out by the host, continues for some while longer to

survive. The crying need, though, is not for a reconciliation of figures but for more facts.

Great differences exist in the quantity of blood possessed by the various mammals used in the laboratory, without compensating differences in the unit concentration of hemoglobin (24), (28), (99), (201). And there are other peculiarities, both individual (172) and specific, which find expression in a widely various resistance of the red corpuscles (130), (235), and differing periods of survival *in vitro* (175), (176). That the rate of blood destruction will vary with the species seems certain, though as yet there are no data upon the point. The fact has recently been brought out that the rate in the individual may be profoundly affected by conditions that lie within normal experience (30), (31). Broun finds that exercise of dogs previously kept to a sedentary life brings about a pronounced breaking-down of red cells which becomes evident because the hematopoietic tissue, being taken by surprise, so to speak, is not at once in a position to replace the loss. He terms the state of affairs marrow decompensation. This decompensation fails to occur when the animals are in training, because the blood-forming tissue is then functionally active as shown by the percentage of circulating reticulated cells; while in addition there is actually more of it present. By contrast, when the body for the time being possesses too much blood, as happens in individuals made plethoric by transfusion, the marrow stops work (168). But to review the literature of blood need as a hematopoietic stimulus is unnecessary. The facts to be derived therefrom interlock with those just mentioned in that they show the existence under ordinary circumstances of a delicately balanced coördination between the rates of cell formation and destruction—a coördination which must often act to conceal great variations in the period of cell survival.

THE SIGNS OF AGE IN RED CELLS. Every normal blood specimen is made up of a mixed population of corpuscles of various ages. In keeping with this state of affairs is the wide range of resistance manifested by the cells on test *in vitro*, whatever the test agent be. Were the same cells susceptible or resistant to all such agents one might be justified in concluding that here are the old and here the young among them. But this is not the case.

From Botazzi (23) to Rusznyak (182) there have been investigators who have believed that the resistance of the cells to hypotonic salt solution yields some indication of their age. But Smith and Brown (202), (203) in extensive studies, found nothing to uphold such a view.

Differences in the staining of the individual corpuscles of amphibians with methylene blue have recently been described, and these differences referred to the age of the cells (204). But a like assumption could be made as concerns differences displayed in tests with any other agent.

Ehrlich (60) thought polychromasia a sign of old age in red cells, but it is now generally recognized to be associated with immaturity and with marrow stress. Polychromatophylic cells circulate normally in a considerable number of species (103). Whether they mature in the blood stream remains to be determined. There is a widespread, if vague, belief that they do, and that this happens also to reticulated corpuscles. But the presence of reticulocytes within the large erythrophages of the spleen of the normal guinea pig can be demonstrated with cresyl blue, so it is certain that some at least are destroyed as such (149). Further evidence of this is to be found in the observation that under pathological conditions which involve the production of reticulocytes in quantity, many undergo destruction by fragmentation soon after their entrance into circulation (170).

**NORMAL METHODS OF BLOOD DESTRUCTION.** Both facts and surmises upon the ordinary fate of the red cell gain a special emphasis from pathological instances, for much of the blood destruction of disease states is consummated by processes that themselves are normal.

*Phagocytosis.* The presence in the spleen of healthy mammals of large cells containing red corpuscles and the products of their disintegration has been recognized for quite half a century. Shortly after the description by Kupffer of the "Sternzellen" of the liver (225), it was noted that in pernicious anemia these endothelial elements act as hematophages (3). They so act in other diseases entailing increased blood destruction (198), (115), malaria, for example; while similar endothelial hematophages are also to be found in the normal bone marrow, in certain lymph nodes, and scattered elsewhere (157). Under pathological conditions, they may be a prominent constituent of the hemo-lymph nodes (230). In healthy mammals, though, the spleen is their special locus. Often, when blood destruction is increased through disease or by experimental means, an immense number of auxiliary hematophages start into being, as it were. They are elements similar, so far as can be seen, to those that normally engulf red cells, but they either have not previously functioned in this way or so occasionally that their activity has passed unnoted. Sections of the spleen may appear tessellated with such elements, gorged with red cells, and crowded against one another; and the size of the organ may undergo a great increase from this cause (117). In

special instances the phagocytosis is more marked elsewhere. It is especially pronounced in the peripheral lymph nodes during the course of experimental anemia due to *Trypanosoma brucei* (27). At the margin of hemorrhages into the tissues anywhere and from any cause, large endothelial elements may be seen which contain red cells. Exceptionally hematophages may appear in the general circulation.

There is no doubt then that phagocytosis is one method whereby some red cells are disposed of normally, and a great many under special pathological circumstances. To not a few investigators it is the all-sufficing means of blood destruction.

The cells which act as hematophages are, save in special instances, of a single kind, though so widely distributed. They are of endothelial origin. Their relationships and general characteristics have often been discussed (66), (5), (184). Their activity in the ingestion of red cells is merely one expression of a widely exercised function; for they will take up with equal alacrity inert particles of many sorts, bacteria, and some of the so-called vital stains. The most striking evidence for their importance in blood destruction is of recent date.

Most of this evidence has been procured by experiments upon birds. In them hematophages are numerous normally (107) and are situate especially in the liver, not in the spleen as in mammals, a difference which Kupffer himself (226), (227) seems to have been the first to emphasize. McNee (125), (126) has repeated the celebrated experiment of Minkowski and Naunyn (132) showing the failure of hepatectomized geese to develop jaundice when the blood is damaged with arsine. He confirms their result, but not their conclusion that the jaundice is essentially hepatic in origin. True, liver activity is responsible for it, but the cells concerned, instead of being those of the parenchyma, are the Kupffer cells, elements the like of which exist in other organs. They ingest and destroy great numbers of the damaged red cells with a formation of bile pigment. When the liver is taken out the elements elsewhere that are capable of functioning as hematophages assume the task of the Kupffer cells; but they are not sufficiently numerous to form bilirubin in the amount required for an outspoken icterus. McNee concludes that the liver of the goose must be looked upon as a double organ, containing as it does two aggregates of cells, parenchymal and endothelial respectively, that have sharply different functions. Recent studies on the formation of bile pigment in hemorrhagic exudates (220), (221), show beyond doubt, — as, for that matter, did Virchow's demonstration of it in old hematomas (224), — that the substance can be formed outside of the liver.

But the most interesting proof, as suggesting a possibly frequent occurrence, is to be found in the derivation of bilirubin from hemoglobin introduced into a circulation from which the liver has been excluded (236).

Lepehne (113), (114), (116) has tested further the relationship of the Kupffer cells to blood destruction. Assuming that the endothelial cells of the liver, if sufficiently engaged with one sort of phagocytosed material might be unable to handle another, he injected collargol into pigeons and geese (116), and, when the endothelial elements had stuffed themselves with it and were presumably incapacitated for the taking up of erythrocytes, he brought about a profuse blood destruction with arsine, after the classical manner. And now, despite the presence of the liver, jaundice failed to develop. The experiment is so engaging that one wishes it were possible to rule out a toxic action of collargol as an explanation of the results.

In rats there are many splenic hematophages. Lepehne (113) finds that after removal of the spleen, the Kupffer cells of the rat liver become active in the destruction of red cells. Pearce and Austin (157) had already noted this vicarious functioning under similar circumstances in dogs, and had found not only the hepatic endothelium engaged in it but that of the lymph nodes as well. And endothelial elements in the hemolymph nodes, as well, may engulf red cells (230). Observations on the occurrence of hemoglobinuria and of hemoglobin-tinted plasma in splenectomized rats (113) require a critical confirmation.

At this writing the precise share taken in blood destruction by the endothelial cells which function as hematophages,—or by the reticulo-endothelial system, as the term now goes,—is much in debate among German students of the icterus problem. Aschoff (5) has well presented the case for the importance of the system. He concludes that it is specifically engaged in the working over of hemoglobin, or else in the elaboration of the ferments requisite therefor. Without laboring any point it may be admitted to possess some such rôle in birds, though whether to the exclusion of other “systems” or cells, notably the cells of the hepatic parenchyma, has yet to be settled. But in the case of many, and not improbably all, mammals some other, or at least some additional, means of blood destruction must be sought. For the number of hematophages normally encountered is obviously insufficient for the task they are supposed to accomplish. The cat has so few of them anywhere that prolonged search may be required for the discovery of a single one (178). Yet the cat possesses an active hematopoietic

tissue. Is one to suppose that in this animal blood destruction does not take place *pari passu* with blood formation?

The nature of the stimulus that leads to a normal phagocytosis of red corpuscles can only be speculated upon. Is the process itself in any sense selective? Corpuscles that have been damaged by a variety of agents tend to lag here and there in organs that contain actual and potential hematophages. The rapidity with which they collect in the spleen is sometimes startling, as is the speed of their subsequent ingestion by cells of the organ. It may well be that they are taken up merely as inert bodies that have been rendered in some way foreign to the organism. On this view the normal ageing of corpuscles would involve changes causing certain of them to come to a stop here and there in the body, with phagocytosis as a secondary consequence. The evidence that the red cells collecting in the spleen of some pathological conditions are "physiologically extravasated" (164), (63), (64), (220) is worth remembering in this connection. Whatever the real case there is no doubt that by some hook or crook of function or morphology the spleen often serves as a sort of midden for damaged erythrocytes.

In the blood plasma of normal animals of several species, antibodies have been demonstrated that suffice to bring about, under highly artificial circumstances, a clumping together of the red cells of the same individual (110), (111). It is tempting to suppose that such agglutinins may cause a lagging of old cells in the blood-destroying organs. But the temperature at which the antibodies cause clumping is far below that of the body, and there is no sign of any such happening in the normal organism.

Circulating antibodies, hemopsonins, that induce a phagocytosis specific for red cells (145), occur occasionally in association with various disease conditions (83), notably such as involve anemia (181). Their presence as a rule attracts attention only when elements containing red corpuscles are found in the blood stream. Usually the hematophages are derived from the fixed elements of the reticulo-endothelial system (223), (16); but occasionally all of the leucocytes, even mast-cells, may ingest erythrocytes (181). It has not been possible as yet to study the condition of the viscera in such instances, with a view to learning whether the phenomenon is the peripheral expression of a general activity. Mention has been made of the fact that when the spleen is taken out of an animal in which hematophages are normally prominent, the endothelial elements of other regions take over the task of ingesting red cells. It is perhaps significant that in the first recorded instance of hemato-

phages in the peripheral blood, their occurrence, as also an anemia, followed removal of the spleen (128). In experimental animals subjected to blood destruction or repeatedly injected with dyes or particulate matter, a not inconsiderable number of the endothelial cells of the liver and spleen, engaged for the nonce in phagocytosis, may come loose in the blood (125), (5). Many of them are so bulky with ingested material as to be sieved out of circulation by the capillaries of the lungs.

The plasma of normal man often contains hemopsonins for the cells of individuals of other blood groups (83); and after transfusion of an incompatible blood, a phagocytosis of red cells may occur in the blood stream of the recipient (150). One may suppose that under such circumstance large numbers of the cells are also engulfed in some of the internal organs. For in the nearly analogous cases of animals injected with serum hemolysins or themselves receiving corpuscles from a different species, there is often a great hemophagocytosis, especially in the spleen (117). And in such cases the process comes about, in considerable part at least, through the action of hemopsonins (145).

*Fragmentation.* Somewhere there must exist in the body, if only for a moment, morphological evidence of the disintegration of every red cell. In several mammalian species possessing few hematophages a search of the organs by perfusion has disclosed a wide distribution of extracellular fragments of erythrocytes (178), still holding their hemoglobin but themselves undergoing further reduction into a fine dust. Similar fragments can be recovered from the circulating blood by differential centrifugation. They are the schistocytes of Ehrlich (58), (59), forms derived from ordinary red cells by subdivision. Can it be that here is a method of destruction worthy of consideration?

The view that corpuscles may normally be threshed to pieces in the circulation is not new (130), nor will it seem strange to anyone who has watched in the living animal, a red cell saddle-bagged at a capillary fork, pulled well-nigh in two, with its bagging portions continually belabored and dragged upon by its passing fellows. Ehrlich thought that the formation of schistocytes comes about as the result of serum changes, and he argued that in the anemias it is purposeful inasmuch as it increases the cell-surface. But the severe occupational strain upon the cells will amply account for the breaking in pieces. When these elements have been damaged in any one of many ways which do not involve immediate destruction, for example, by a serum hemolysin (140), or by the poisonous principle of certain mushrooms (161), or by heat, as during burns (160), (84), (105), an almost immediate consequence is

fragmentation in the blood stream. The changes are tersely and adequately described by Ponfick (160), (161) who was perhaps the first to follow them. Many of the cell fragments produced under such circumstances give up their hemoglobin. As a rule the cell débris rapidly leaves the circulation, lodging in the spleen and elsewhere. Certain of the normal corpuscles of amphibians can be stained while yet circulating, and cells of this sort show a special tendency to fragment (204).

Schistocytes are prominent in the blood picture of many anemias, and are perhaps the more noteworthy because they have given rise to so little speculation. Ashby (7) has published a significant picture of the blood of a recently transfused patient with pernicious anemia, in which the erythrocytes of foreign, that is to say healthy, origin appear sharp-outlined and perfect, in contrast with the irregular, pale or dark, rag, tag and bob-tail corpuscles that are the patient's own possession. Incidentally the picture aids one to understand the strikingly long survival of such transfused cells in patients giving every evidence of continued severe blood destruction. It is, in part at least, a survival of the fit.

May not the schistocytes of the anemias be put forth as such by the bone-marrow? Granted, as concerns some of those found in connection with a marrow so abnormal as that of pernicious anemia. But this does not hold for the cell fragments encountered during regeneration from experimental anemia brought about by bleeding. Under such circumstances large numbers of corpuscles of soft, jelly-like consistency appear in the circulation, many of them soon breaking up into fragments (169). The fragments tend to accumulate in the spleen, but are to be encountered elsewhere as well, though not in the marrow, significantly enough. During the disappearance of compatible blood from healthy animals made plethoric by transfusion, schistocytes become numerous in the circulation and collect in the organs.

If any considerable number of corpuscles are normally threshed to pieces in the blood stream, then an increased functional wear and tear, as when the circulation is quickened, may lead to an increase in the destruction. Poikilocytes and schistocytes are said to appear in the blood of individuals going to high altitudes (60). Mention has been made of the fact that in exercised dogs blood destruction is much more considerable than in sedentary animals (30), (31). An increase in the circulating schistocytes has not been demonstrated. In normal mammals the number of fragmented forms in the blood stream is never great, nor is the accumulation in the spleen impressive.

It has been suggested on histological grounds that the ultimate fate of red cells may be a phagocytosis in the spleen, following fragmentation (2). Lepelne (115) has studied the character of the content of the splenic hematophages in human beings. In individuals dying with evidence of toxic or thermic blood injury great numbers of hemoglobin-containing fragments were present in the hematophages, whatever the situation of these latter, and a lesser number of such fragments was also free in the blood. Lepelne believes that he has discovered a new method of blood destruction, *erythrorhexis* within phagocytes; yet his evidence accords better with the view that fragmentation took place outside of these elements. It is known so to do after toxic and thermic injuries, as already mentioned a page or so back.

The conception that cell destruction in the anemias comes about in considerable part by fragmentation soon after the cells leave the parent tissue involves the view that this tissue in repairing an anemia has to cope not only with the original difficulty responsible for the inferiority of its present yield, but with extra losses consequent on the inferiority. This unfortunate state of affairs probably exists, no matter what the mode by which worn-out cells are disposed of. For it is scarcely to be expected that the corpuscles manufactured under pathological conditions will withstand the exigencies of circulation as long as normal ones. All of which is to say that cell-mortality will continue high until an adequate type of corpuscle is available. The point has received too little recognition in attempts to comprehend the course of blood repair.

The phenomenon of hemolysis in the test tube is so impressive as to have suggested to many observers that normal blood destruction must be effected in some such way. Italian and French hematologists especially have inclined to such a view. They appear to have tacitly accepted the inadequacy of phagocytosis as an explanation of the fate of the corpuscles, and they have been influenced in opinion by the studies of their compatriots on hemolytic icterus, and the favorable results of splenectomy in several blood diseases. The hypothesis according to which blood is normally hemolyzed was first formulated long ago, by Botazzi (23). According to Banti (12), (13), whose conceptions have been widely adopted, there exist in the body "hemolytic organs," of which the spleen is the most important, which may be excited in various ways to great activity and are so excited in the hemolytic icterus above referred to. Many experiments are on record in which normal hemolysins feature, and some demonstrations as well of free hemoglobin in blood from the splenic

vein; but the difficulties of interpretation are great. Injections of splenic extract are said to lower the number of circulating red cells. The whole subject has been reviewed by Pearce and Krumbhaar (158),—who have made many observations in connection with it,—and recently by Eddy (57). At the present time the thesis that hemolysis is concerned in normal blood destruction must be looked upon as not proven.

To sum up, only two normal methods have thus far been discovered whereby worn-out red corpuscles leave the circulation, namely, phagocytosis and fragmentation. The amount of phagocytosis normally going on in the body is often insufficient to account for the disappearance of many cells whereas a constant slight fragmentation throughout the circulation will readily do so. Both processes may quite possibly be of little consequence as compared with some other, unrecognized as yet. One is privileged to believe as one wishes about the matter, but scarcely to draw conclusions.

Phagocytosis and fragmentation of the corpuscle have been discussed at length because they are processes natural not only to the healthy, but to the diseased organism in which large quantities of blood are rapidly broken down. They serve often in this latter connection as the means of first resort. But there are a host of pathological conditions in which destruction of the red cells comes about by methods essentially morbid and frequently of extraneous derivation. These methods will be but briefly dwelt upon.

**ABNORMAL METHODS OF BLOOD DESTRUCTION.** Many forms of injury to the red cell that are encountered in man, under present industrial conditions, are so bizarre as almost to rank with the wantonly experimental. They are not enconced in the life of the species like injury by the malarial parasite and by bacterial organisms. And they need be mentioned here only as they throw light on physiological laws. This they sometimes do. But it is the weakness of much of the current experimental work on blood diseases that it is carried out with injurious agents the effects of which on the red cell and on the animal body can only be regarded as highly artificial.

*Hemolysis.* The chemical and physical agents which bring about a liberation of hemoglobin from the corpuscles are legion; and various are the ways in which they compass the result (235). Several attempts have been made to follow, through the darkfield microscope, the morphological changes incident to hemolysis. They have been so far successful as to have shown that the changes are not always the same (53), (186). The resistance of the cells to any one hemolysin, as in-

dicated by the freeing of pigment from the corpuscle, is often no index to that of others (183), (104). What agent then shall one take as an indicator of the age or condition of the cell? Manifestly the facts give no warrant for taking any. Yet it is current practice to choose an hemolysin for clinical tests of cell resistance, usually one that is readily available and easily manipulated. And our ignorance justifies the practice. Cell peculiarities have been demonstrated thereby in not a few conditions, and although their meaning awaits elucidation, the discovery of them has aroused speculation when it has not aided diagnosis.

That hemolysis may on occasion take place in the animal body has been realized ever since the disastrous seventeenth century efforts to invigorate the old and sometimes wicked with blood from the young and sufficiently innocent. The literature of the time contains many succinct descriptions of the events which followed the giving of a wrong strange blood (109). The studies of Bordet and Ehrlich on serum hemolysins rationalized these happenings and set a throng of investigators upon the idea that the activity of such antibodies would explain blood destruction in general (185). Unfortunately, the existence in the normal human organism of isolysins and isoagglutinins was not recognized for several years (112), (137); and many findings which seemed significant have now been traced to them. At the present day the destruction of one disease at least, paroxysmal hemoglobinuria (54), is known to be produced by a serum hemolysin having the character of an antibody; and in several other maladies in which there are signs of a great breaking down of corpuscles, notably in pernicious anemia (174), (242), (228) and some forms of hemolytic icterus (46), (212), auto-hemolysins have been encountered. The effect of isohemolysins is only too frequently witnessed in transfused cases. No further comment would be called for in this connection save for a curious development of a sort essentially modern. The technique of transfusion methods has been pushed to perfection without a proportionate understanding of the results of the procedure; and in consequence there are now on record not a few instances in which, through frequent transfusions, immunization of the human creature against bloods previously compatible has been unwittingly accomplished (22), (208). Often the immunized individual can now no longer be helped by the transfer of corpuscles from anyone, but is greatly harmed instead. The newly developed lysins may or may not be demonstrable *in vitro*.

Under pathological circumstances substances potentially hemolytic but

normal to special organs or tracts may escape from their usual environment into the general circulation and damage the red cells. The bile of most species is hemolytic, by reason of the cholates that it contains (119); and the anemia so often associated with prolonged jaundice from obstruction has been attributed to lysis by bile salts, though, as we now know, the plasma acts to prevent this (18), (119), (199). The anemia of nephritis, as of certain other diseases, is not inconceivably due in part to lysis by retained metabolic products. Hemolysins have been demonstrated in necrotic tissues (232); and blood destruction in some patients with large necrosing tumors may conceivably be caused by such bodies. Chronic poisoning with toluylenediamine results in the formation of hemolytic substances by autolysis (98), (127). The suggestion that fatty acids may be responsible for a lysis of cells in patients has led to much work (101); but it is not supported by the most recent findings (52). The need for a rational therapy of pernicious anemia, coupled with the evidence for hemolysis in the disease, has led to a wide extension of knowledge as regards lytic agents which, one by one, through successive waves of investigation, have been proven guiltless in the matter (153).

Banti's view (12), (13) that blood destruction is caused by a hyperactivity for hemolysis of certain organs, notably the spleen (71), (73), (74), (75) has been discussed in its relation to normal blood destruction. Whether the hemolysins which can not infrequently be separated out of the tissues (69), (21), (147), (148), (233), (234) play any rôle in body processes is dubious, to say the least. Hunter (94) is largely responsible for the stimulative idea that blood destruction in pernicious anemia comes about through the action of a lytic substance absorbed from the intestinal tract. The finding of Bunting (43) and others (120) that blood pictures much like those characteristic of the disease may follow upon the repeated intravascular injection of hemolysins; Tallqvist's (210) observations on the anemia sometimes developing in individuals who harbor the tapeworm *Bothriocephalus latus*; and the demonstration of the permeability of the intestinal wall, not only to some of the simpler hemolytic poisons (96) but to serum antibodies (67), are alike encouraging to such a view. Yet there are investigators who maintain that pernicious anemia is not a disease of blood destruction at all. They pit isolated facts or ingenious hypotheses against the mass of data which attests to a havoc among the red cells.

The discovery that certain microorganisms will produce lysins (hemotoxins) (213), (214), (121), (163), (161) under appropriate conditions

has a potential bearing not only on the anemias of obscure origin but on the serious blood changes that occur in many infectious diseases. In most such instances we do not yet know whether an increased destruction or a lessened formation of cells, or a combination of both, is to be blamed. In the average case of scarlet fever, for example, a drop in the red count of about one million cells is said to occur (90), and with that said, knowledge upon the matter has been nearly summed up (108). Yet it should be possible, by methods now available, to determine where in the blood cycle the trouble occurs.

Some of the hemotoxins, those truly deserving the name, behave like genuine toxins, inducing in the animal a formation of antibodies which may on occasion have a high titre (213), (214). The recent work of Bull on the toxin of *B. welchii* (40), (41), (42) furnishes a case in point. The nature of many other hemotoxins is only vaguely apprehended. There is good reason to suppose that the malarial organism (15), (29), (244) and numerous other animal parasites give off substances that cause lysis (209), (210). But the literature on hemolysins of extrinsic origin,—a literature of stupefying dimensions,—cannot be reviewed here. Work with these agents has not of late greatly furthered an understanding of the laws of blood destruction within the organism. In 1877 Ponfick (160), describing of the effects of mushroom poisoning in dogs, set forth in a couple of paragraphs nearly all that we now know of the ways in which red cells go to pieces when acted upon within the body by an hemolysin. He observed lysis and fragmentation occurring together, and a local deposition of the cell debris with subsequent phagocytosis of it and of cells still intact.

The failure to demonstrate serum or other hemolysins in clinical conditions in which there exists good reason to suppose them active is evidence rather for the inadequacy of current methods of test than for conclusions on the state of affairs within the body. For example, an amount of serum hemolysin, which suffices to lake but a few cubic centimeters of blood *in vitro* may, when introduced into the organism, give rise to great destruction (139). This comes about in part through a transfer of the hemolysin from cell to cell, and in other part (135), (136), (138), (139), through an increased fragmentation and phagocytosis of corpuscles to which they become liable without any liberation of pigment having taken place. Furthermore, there can be no doubt that an injury insufficient to produce hemolysis under test-tube conditions may bring it about in the animal body. The development of methods which

will enable one to recognize *in vitro* when red cells become non-viable rests as an urgent task upon hematologists. At present workers employ, of necessity, the crudest possible criterion, namely, lysis.

There are yet other sources of difficulty in the demonstration of hemolysins in disease states. Some of them never enter the general circulation. The one which is responsible, presumably, for the anemia associated with the broad tapeworm (*Bothriocephalus*) disappears from the blood of animals within 15 minutes after injection, as experiment has shown (209); and yet it does not fail to exert for a much longer period its destructive influence. Some agents give rise to hemolysis only as the result of changes they induce in the tissues (98). There are modifying serum factors and others of dilution and proportion which are not taken into account in test-mixtures as ordinarily set up,—factors which vary greatly from individual to individual. But it is unnecessary to enlarge on our inability to copy body conditions.

**HEMAGGLUTINATION.** Landsteiner (110), who first demonstrated the presence of autohemagglutinins in the plasma of normal animals, showed that these bodies have the same essential characters as the iso- and heteroagglutinins, but are much weaker, and, as already mentioned, act to cause a clumping of cells only under highly artificial conditions. Agglutinins have also been found in extracts of the normal corpuscles of some species (102), (171). The question arises whether under pathological conditions such auto-antibodies ever bring about blood destruction. There are facts to suggest that they do:—In diseased human beings they are occasionally of high titre (89), (48), (241) and are found in association with anemia. Yet it is not always certain that the anemia may not be a cause for their occurrence rather than an effect of it. For autoagglutinins appear in animals rendered anemic by bleeding (170), as also when blood destruction is increased by the repeated injection of compatible corpuscles (180), (171). It is claimed that when blood kept outside of the body for several days is returned to the individual whence it came, autoagglutinins develop (121). The fact may be granted but its interpretation remains uncertain. For the temporary removal of the blood entails a temporary anemia such as may itself lead to an appearance of agglutinins.

Isoagglutinins are responsible for some of the untoward effects of the transfusion of incompatible blood (150), (151). Certain disease-producing bacteria give rise to agglutinins effective against the corpuscle (244). Serious changes in the organs as a result of hemagglutination

have been described (70), (154), (155). The nature of these changes is such that any considerable participation of agglutination in pathological blood destruction would surely have been discovered ere now.

CELL DESTRUCTION THROUGH PIGMENT CHANGES. On pathological occasion the hemoglobin of erythrocytes may be so combined with or altered that the cells become more or less useless as oxygen carriers. The combination may be reversible, and, in one such case at least, that of carbon monoxide poisoning, the affected corpuscles continue to circulate until restored to their normal condition (79), (146). When a permanent change has been effected, as to methemoglobin, they may rapidly leave the blood stream although still intact (207). A profound anemia may thus develop within a few minutes. The fate of the changed pigment has not been traced. Cole (49) and others (45) have brought out the fact that infection with the pneumococcus can lead to some formation of methemoglobin. Usually this is not found free in the serum, though Van den Bergh and Engelkes (222) have described both methemoglobinemia and sulphmethemoglobinemia accompanied by hemolysis. In many forms of fulminant blood destruction hematinemia has been noted (193), (194), (195) as the result of changes in the hemoglobin after it has left the corpuscle.

THE INFLUENCE OF CONGENITAL PECULIARITIES ON BLOOD DESTRUCTION. In an occasional human being some of the erythrocytes habitually put forth by the marrow are disc shaped (55), (56) or even of an attenuated sickle shape (61), (85); and there may be a family tendency to the condition and an anemia associated with it. Other individuals are born with a tendency to jaundice of hemolytic character (212); and here again a family influence is often visible. The peculiarity may assert itself merely by the presence of slightly more bilirubin in the blood serum than is normal (*cholémie familiale simple*); or again it may lead to pronounced jaundice and anemia persisting throughout a long life. Autoagglutinins and hemolysins have been demonstrated in individual instances (46). A noteworthy, almost unique, character of the erythrocytes is their lessened resistance to lysis by hypotonic solutions (44). Resistance of the sort is remarkably uniform in most diseases and in normal states as well, though, strangely enough, it is enhanced in the obstructive forms of jaundice.

The conclusion cannot be avoided that some congenital factor is active in all the conditions mentioned. But the nature of the factor, or factors, affecting the corpuscles has thus far eluded determination. There is evidence that plasma abnormalities may be the cause for sickle-

shaped red cells, and some observers would lay the various forms of family cholemia to a liver derangement (17),—though a marrow abnormality affecting the character of the cell output seems more likely. Whatever the trouble its ultimate results are in one respect at least often the same. There is increased blood destruction with splenic enlargement. The steps in the process of destruction have not been followed. Banti's explanation—that there is a splenic hyperactivity leading to hemolysis—has already been discussed.

**CELL DESTRUCTION BY EXTRAVASATION.** Erythrocytes confined between ligatures upon a vessel break down much more slowly than do others escaping into the tissues (192). Tissue ferments have been held responsible for the difference. It is certain that the great majority of the red cells of hematomas do not regain the circulation but disintegrate on the spot. Kretz (106) suggested many years ago that in some obscure cases in which the liver contains an abnormally large amount of blood pigment, repeated small hemorrhages will account for the finding. The anemia of severe cases of purpura is clearly traceable to the repeated local extravasation and disintegration of blood. Whether there are other anemias consequent on long-continued occult hemorrhages within the body has not been ascertained.

The course of events in hematomas is one of the oldest themes in modern pathology (224). Recently the alterations in the blood pigment have been studied, with improved methods, by Van den Bergh and his associates (219), (220). They emphasize the similarity to changes taking place in the spleen in some of the blood diseases. Pribram (164) had noted that in chronic passive congestion of the spleen there is a marked local breaking down of blood; while Eppinger (64) had laid stress on the vascular peculiarities of the organ in pernicious anemia, advocating the view that the destruction of red cells results from what is in effect a constantly recurring extravasation into the splenic pulp. And now Van den Bergh has brought chemical evidence for a "physiological extravasation" of red cells, not alone in pernicious anemia but in other conditions.

**DESTRUCTION AS AFFECTED BY PLASMA CHANGES.** According to Hamburger (81) and the idea has become a familiar one to students of the chemistry of the blood—however simple a plasma change may be, its consequence is a change in the chemical constitution of the red blood cell. There can be no question but that many plasma changes which take place within the body must make for or against the survival of the corpuscle, if not by causing interior alterations, then by acting from

without. The fluid of the normal blood acts to protect the cells against mechanical insult, and from the effects of many hemolysins (235). But may not the highly abnormal, only slightly viscous plasma of pernicious anemia (144) fail in some degree to ward off mechanical harm to the cells during their incessant round of the body? A decreased power of the plasma in this disease to protect red cells from hemolysis by soaps has lately been described (47); but that the power itself is of practical moment has now to be proven. An attempt has been made to group with the various forms of congenital jaundice those blood states characterized by the presence of sickle or disc-shaped cells as well as the others in which autoagglutinins or hemopsonins have been found, and to attribute all to plasma changes (229).

**THE RATE OF PATHOLOGICAL BLOOD DESTRUCTION.** The possibility that the destruction of red corpuscles may sometimes slacken in disease has been scarcely considered, so generally is it hastened. Yet there are data to show that in rabbits transfused and having need of the blood thus provided this lasts longer than in normal animals (26). The finding may possibly help to explain the protracted survival of transfused cells in some human beings (7), (8), (9). The assertion has recently been made that the degradation of hemoglobin by the liver can be delayed by poisons acting upon the organ, and that a polycythemia then ensues which is the result of decreased blood destruction (88). Almost the whole of previous knowledge speaks against such a happening.

The increased blood destruction of many pathological states is accomplished, as it will do no harm to state again, by methods that are normal in themselves. Hunter assumed as much in his studies of the fate of the corpuscles in dogs made repeatedly plethoric by transfusion (93). He observed that an ability to break down the blood with speed was acquired by the animals, a finding confirmed by later workers (25), (87). The destruction, in so far as it is brought about by antibodies directed against the alien cells, must be considered pathological. But the demonstration that exercise hastens corpuscular disintegration in normal animals (30), (31) suggests the thought that, in many diseases unrelated directly to the blood, the physiological apparatus concerned in the destruction of its cells may be affected.

Current estimates upon the pace of cell destruction in disease are based upon the same insecure premises and data as are those for health. The amount of urobilin in the stools has been assumed by many workers to yield an index to it (62), (243), (167); and certainly there are

clinical observations attesting to a usefulness of the substance in this connection. But urobilin is derived from bilirubin, which, as we have seen, may not represent destroyed blood quantitatively; while furthermore the amount of urobilin which can be got from normal stools by present methods is far below that which should be yielded by the bilirubin turned into the intestines (62). Eppinger and Charnass, admitting that this prevents inferences on normal blood destruction, hold nevertheless that calculations from the quantity of urobilin found under pathological conditions can at worst lead only to an under-estimation of the number of cells destroyed. This quantity is so great in some cases of congenital hemolytic jaundice that a turnover of the entire amount of blood once in every two or three days would be required to account for it (62). A recent reviewer, discussing the possibility of a similarly swift destruction and replacement in pernicious anemia, finds it so far out of the question as to reduce to absurdity the general reliance upon urobilin figures (239). Yet when one considers how rapidly blood broken down within the body may be replaced, how little the total quantity sometimes is in the diseases just mentioned, and how widespread and active the hematopoietic tissue, this seems not necessarily to follow.

The appearance of punctated cells in individuals given small doses of lead by mouth has been utilized in a study of the period of survival of corpuscles in human beings with malaria (50). Some of the punctated cells survive for 2 days at least. The method is highly limited in application, needless to remark.

A destruction of the greater part, if not of the whole of the blood can be brought about before life is extinct by the use of physical and chemical agents. But these agents and the physiological situations they induce have little interest in the present connection.

**DISPOSAL OF THE CELL MATERIAL.** The capabilities of the organism to deal with damaged corpuscles are very great. Cells that have been injured by experimental means usually leave the circulation practically at once. It might, indeed, almost be set down as a general rule that their continued presence in the blood stream means that serious destruction is still going on, and that the animal will die if it be not soon checked. This rule would fail to hold in connection with the damaged corpuscles of disease states. The distorted or fragmented cells of many anemias, unlike those occurring in consequence of the action of poisons of extrinsic origin, must certainly go the round of the vessels for some time, since if they disappeared promptly there would often be little blood left. And

corpuseles carrying the malarial plasmodium may function until their substance had been almost wholly replaced by the parasite.

The morphological changes undergone by phagocytosed red cells have often been followed. The view is widely accepted that normally the hemoglobin derived from them is passed on from the spleen to the liver. This involves the assumption of some sort of intracellular hemolysis. But the histological evidence is wholly against such an occurrence. The red corpuseles within hematophages are never visibly decolorized, nor do they merge into larger hemoglobin-tinted globules such as form after the ingestion of cells already hemolyzing (177). Instead, cell fragments of various size are regularly seen, all of them smaller than the ordinary erythrocyte. They are at first rounded and give the staining reactions for hemoglobin, but are sometimes distinguishable by other means from fragments of extracellular origin (178). They undergo a gradual conversion into angular brown granules of hemosiderin, and one gains the impression that the changes take place at a slow rate. The tardy utilization of the iron deposited in splenic phagocytes, as compared with that in the hepatic parenchyma, is in keeping with this impression (142). Biliverdin has been recognized in the hematophages of birds (5).

The normal fragmentation of red cells progresses until they are reduced to a fine, hemoglobin-containing dust. Some of the fragments and dust lodge here and there, especially in the spleen (178). But where and how the ultimate separation of hemoglobin and cell fabric takes place remains to be discovered. Not improbably it occurs throughout the circulation, and the accumulation of schistocytes in the spleen is incidental to the peculiar vascular arrangement of this organ. The fact that bilirubin can be formed from blood pigment almost anywhere in the body fits in with such a conception.

Some part of the cell-fabric, or stroma, has an immunological peculiarity (185) and might conceivably be traced. It has been separated from the cells in the test tube (11). But the conservation of the stroma substances would seem to be of little importance to the organism. McMaster and Haessler (122) were unable to deplete the body of them to such extent as to interfere with the formation of new erythrocytes. It is a common observation that the production of stroma regularly outruns that of hemoglobin during recovery from the anemia of hemorrhage. The statement has been made that such repair may be hastened, both as regards cell number and hemoglobin percentage, by the injection of lipoids derived from the corpuseles of an animal of another species (100).

Gross lysis of cells in the blood stream brings with it a large train of pathological consequences. With the more remote we are not concerned. Observers have likened the immediate shock to that of anaphylaxis (20), (22) and some believe that it is referable to similar causes. Blood crises characterized by the appearance of immature cell forms in circulation, and closely resembling those of pernicious anemia, occasionally follow upon the transfusion of an incompatible blood (152). The factor responsible for them has yet to be identified. Free hemoglobin seems not to be toxic (14), (200), save perhaps in occasional disease instances. Artificially prepared globin, though, may cause serious disturbance, at least in experimental animals (190). And the stroma from the cells gives rise, in addition to fever, to serious disturbances in some species (14),—not in others (20),—by lodging here and there and mechanically obstructing the blood stream. It is the better enabled so to do because it induces clotting (118). It collects for the greater part in the spleen and lungs. When blood destruction has been brought about with toluylenediamine in splenectomized animals the “shadows” of hemolyzed cells circulate for a much longer time than is the case in controls not deprived of the spleen (97).

Free hemoglobin, hematin and bilirubin may all be met with in old hematomas; and they may be associated together in the large spleens of patients with some of the blood diseases (220). The formation of bilirubin in the perfused normal spleen has recently been described (65). That the pigment, from damaged cells accumulating in the organ may undergo the same changes as in hematomas one can readily believe. Van den Bergh's view that the destruction of corpuscles in pernicious anemia comes about by a “physiological extravasation” into the splenic tissue is based on this assumption.

Free hemoglobin has not been demonstrated in normal plasma, although in some species of animals (217), notably in man, bilirubin is present in a concentration which permits quantitative observations (91), (218), (220). Injected hemoglobin is rapidly removed from the blood stream, notably by the liver. Crystals of it have been recognized in the hepatic parenchyma of healthy animals (32), (86). That it cannot be immediately utilized again, as such, for new-formed corpuscles is indicated by the tardy changes in the color index of the blood of animals injected with it when they are putting out abnormally pale cells to repair losses from hemorrhage (122). Indications exist of intermediate stations (*Zwischenstationen*) (191) where it is worked over. The fact that it is manufactured with more difficulty than the rest of the cell has already

been referred to. In view of these facts there is nothing surprising in the circumstance that the body possesses an excellent arrangement for the retention of the more important part of such pigment as may accidentally be liberated from corpuscles (141). Considerable quantities must accumulate in the plasma before any escapes into the urine (51); and the liver acts to prevent the necessary accumulation by swiftly removing it from circulation (10), (156). In rabbits given a specific hemolysin, more than one-half of all the blood may be destroyed within 48 hours and yet no hemoglobinuria occur (140). But in human beings long subject to blood destruction the pigment may appear in the urine after injections of it which in healthy individuals would have no such result (200). Either the liver or the kidney may conceivably be at fault in such instances.

In animals rendered anemic by blood destruction within the body the loss is repaired more rapidly and satisfactorily than when depletion has been accomplished by bleeding (95), (166). The pace of recovery depends, within limits, on the amount of hemoglobin available. Not only are cells of better quality put out when this is abundant, but there then occurs an extension of the red marrow, so that soon the body comes to possess more blood-forming tissue with which to meet the situation (122).

The prevailing view on the normal fate of hemoglobin is that it is broken up into globin and hematin, which latter in turn yields hematoidin,—thrown off in the bile as bilirubin,—and iron-containing materials of uncertain chemical constitution which are retained (1), (235). When an unusual amount of the iron-containing stuff collects in the body it can often be recognized by microchemical reactions. Recent work makes one doubt whether that is all there is to the matter. Two sorts of bilirubin have now been distinguished, one formed through liver activity, and another arising without intervention of the organ (220), (221); and certain suggestive anomalies of pigment metabolism have been described. There is a condition of congenital origin in which hematoporphyrin (78), nearly related to hematin, is excreted in the urine in large amount, and in which, moreover, hematin itself is found in the blood, together with bilirubin (196), (197). In some diseases iron has been found in the plasma in such a state that it may be readily demonstrated, whereas in others it occurs in "masked form" (220). Hematin (194), (68) may circulate in such quantity as to color the plasma a yellow-brown (194). The obdurate character of this pigment had led to the supposition that it cannot be an intermediate stage in the normal

degradation of hemoglobin (33), (34), (35), (36). The truth would seem to be that at present one can only make conjectures about the course normally followed in the degradation of hemoglobin. The routes for its destruction under pathological circumstances are many-tracked. The relation of the hemoglobin of the blood to myohematin, to the hemofuscin of hemochromatosis, to the pigment of brown atrophy, and to the yellow or brown material laid down in certain organs as the body ages, has all to be worked out, as has the connection of the blood pigment with the urinary pigments and with the iron normal to the cells of the tissues in general (80), (19), (4).

Repeated attempts have been made to work backwards up the trail of the hemoglobin derivatives to the sites, if not of blood destruction, then of the pigment metabolism. The results of analyses of the organs for iron have given rise to interesting and diverse hypotheses on the fate of the erythrocyte (4), (191), (115). But numerous facts point to a need for caution in this connection. The bone-marrow of normal man, a tissue not greatly concerned, so far as is known, in the destruction of corpuscles, yields the microchemical reactions for iron more often than does any other tissue (191). The iron derived from hemolyzed corpuscles is not distributed like that from phagocytosed ones (141). Furthermore, local injury without blood extravasation may result, under appropriate conditions, in a local accumulation of hemosiderin (179). And in hemochromatosis, a disease characterized by the extensive deposition of iron-containing pigments, there is no evidence whatever of increased blood-destruction but much that is practically conclusive against it (131), (205), (92). The peculiar abundance of hemosiderin in the hepatic parenchyma of patients with pernicious anemia can scarcely attest, as so many think, to a portal disintegration of erythrocytes. For the repeated injection of small quantities of hemoglobin into the systemic circulation will bring about a like condition (123). And how is one to reconcile the idea of a "physiological extravasation" of blood in the spleen during the course of pernicious anemia with the fact that the iron content of the organ proves frequently to be less than normal (194)? All this, and more, tends to weaken inferences from iron-distribution. Nevertheless, it may serve to confirm impressions gained by other methods. For example, the iron content of the spleen and liver is such as to support the belief that these organs are important in the conservation of blood pigment. The ferruginous material freed and stored during experimental blood destruction is drawn upon in the subsequent repair (142).

## COMMENT

Few generalizations can be gleaned at the present time from the confused multitude of observations upon blood destruction. But this much at least is sure: A disintegration of red corpuscles takes place constantly, in sickness and health, and the body has often to meet new demands and situations connected therewith. These are usually coped with successfully, in so far as concerns the immediate task of accomplishing additional destruction, when that is necessary, or disposing of the products of a breaking-down unprovoked by the body. But for such emergency purposes other mechanisms besides the normal must frequently be called upon. It is the interplay and substitute activities of these reserve mechanisms which make conclusions so difficult upon the normal as well as the pathological course of events. Given the many heterogeneous phenomena of blood destruction, representing reactions of the body to disease and to experimental procedures, the task of the investigator is to determine the precise elements responsible for such physiological combinations and permutations. And it is not as if the elements concerned had fixed dimensions or values. When one is excluded from a rôle in the organism, others assume an unwonted importance and the net result is the same in all save details of execution.

The reader who has come thus far will not fail to have noted that much of current theory upon blood destruction attests to the theorist's abhorrence of a vacuum. If the reviewer, in discussing various conceptions has seemed to blow both hot and cold as regards them, this is no more than the facts themselves do. And yet the profusion and diversity of these facts already gained is such that they will surely suffice to embody and extra-illustrate many times over the complex principles regulating the fate of the red cell,—once these principles stand clear.

*Note on the bibliography.* Much of the literature on blood destruction is shut away behind titles and within subjects that a reviewer will naturally pass by. In that here cited less regard has been had for priority, and perhaps even for significance, than for aptness in illustration, and usefulness to the enquirer. A selection, and in the nature of the case a relatively small selection, has been made from the great mass of titles.

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## THE ETIOLOGY OF RICKETS

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"The history of rickets is that it has been enriched by a wealth of new hypotheses but few new facts."<sup>1</sup> This statement made in 1908 is no longer true since in the past four years facts of the greatest value have been discovered.

In 1650 Glisson (2) established rickets as a clinical entity, which received yet sharper definition, in the latter part of the last century, as cretinism, chondrodystrophy, osteogenesis imperfecta and scurvy were disentwined from the supporting stem through the work of Virchow, Parrot, Porak, Kirchberg, Marchand, Stelling, Kaufmann, Barlow and others. In 1885 Pommer (3) established on a firm foundation for all time the pathology of rickets through magnificent histological studies which were corroborated and extended by v. Recklinghausen (4), Schmidt (5) and Schmorl (6). The past four years have been marked by inroads into the etiology of rickets, and the past two years by the beginnings of an insight into the disturbance of the salt metabolism which constitutes the disease.

Rickets is a disturbance in the metabolism of the growing organism of such nature that the salt equilibrium, in particular as regards the calcium and phosphorus, in the circulating fluids is disturbed, and lime salts no longer deposit in the bones. Lime salts may not deposit because the ionized calcium in the blood is low, or because the ionized phosphate is low, or because both are low. When, however, the calcium in the blood is low, the formation of new bone and the destruction of old calcified bone (umbau) is greatly accelerated, and the pathological process takes on a distinctive character. But no fundamental differences exist between the low calcium and the low phosphorus forms of the disease. Increasing knowledge concerning rickets has made it necessary to broaden the view in regard to the characteristic pathology and admit to the disease all disturbances in metabolism in which lime salts cease to be deposited in the bones and cartilage. The first

<sup>1</sup> Quotation from Albu and Neuberg cited by Findlay, Paton and Sharpe (1).

detectable signs of rickets are probably a diminution of the inorganic phosphorus or calcium of the blood.

**THE OCCURRENCE OF RICKETS:** *The geographical distribution.* For a complete presentation of this subject the reader is referred to the splendid article by Palm (7) and to the recent book of Dick (8), and to the historical sketch by Findlay (9) and to the book by Dick for the evidence concerning the existence and distribution of rickets in ancient times. Here one should mention a few facts. Rickets occurs chiefly in Europe and North America. It is a disease found in cities. It is most prevalent in those nations whose wealth and industrial development have brought about most fully the substitution of artificial conditions of living in place of the simple conditions which nature intended. Wherever civilization of this artificial character establishes new contacts, rickets begins to appear. The disease has appeared in India where the designs of nature have been interfered with by religious customs to be mentioned later. The disease never occurs among peoples living under natural conditions. Savages may starve and may become the victims of pestilence, but they do not develop rickets. The disease does not occur in the native parts of Africa and is exceedingly rare in China and Japan. It occurs rarely in the tropics, and is rare in the Arctic regions.

*The prevalence among animals.* Jost and Koch (10) state that rickets is a common disease among pigs, puppies, lambs and kids, but less common among colts, calves and rabbits. It manifests itself with comparative frequency among carnivorous animals and also among monkeys in captivity. The striking facts concerning the occurrence of rickets among animals are as follows: The disease appears only among those animals which man has been able to make captives or slaves and upon which he has been able to impose successfully such artificial conditions of environment and diet as he has developed for himself in his struggle for existence. The disease never develops among animals living apart from man and probably cannot develop in animals or in man in the wild state. Hansemann (11) points out that the cat, in contrast to the dog, never develops rickets. He gives as his reason the fact that the cat, though tamed, can never be made to relinquish the habits natural to the species. Apparently rickets develops frequently in the monkey when he is in the zoölogical garden, but never when he is at liberty. Rickets can be produced in the domesticated rat but only by unusual means. It is probable that the rat is not readily susceptible to the development of rickets.

*The frequency of occurrence.* Inasmuch as rickets can be determined by clinical means in only a fraction of the cases, it is impossible to obtain exact information concerning its frequency of occurrence. One can say that rickets is so common in the large cities of America and Europe that few children among the poorer classes are untouched by it. Data bearing on this subject will be found in the section devoted to the seasonal variation in rickets.

*The occurrence in utero.* For a complete discussion of the controversy concerning this question, the reader is referred to Wieland (12). The most violent advocate of the congenital origin of rickets in recent times has been Kassowitz (13). He believed that rickets had a fetal origin in a large percentage of cases. The clinical evidence consisted in soft areas in the bones of the head, enlargement of the fontanelles and of the lines of suture, prominence of the bosses, enlargement of the costochondral junctions, and protuberance of the abdomen. The anatomical evidence need not be recited, for Kassowitz's understanding of the pathological changes and processes in rickets was such as to lead him to confuse the normal condition with the rachitic, and to make him unable to separate rickets from syphilis.

The best evidence against congenital rickets has been brought forward by Schnorl (6) who investigated the ribs and long bones of the extremities of more than 100 infants born either at term or prematurely, without once finding evidences of the disease. Pommer (3), Lentz (14), Tschistowitsch (15) and Wieland (12) have shown conclusively that the spots of defective ossification in the skull in new-born infants have no connection with rickets.

The only way in which proof of the existence of congenital rickets can be obtained is by histological examination of the bones. Whatever reliable evidence comes from that source indicates that rickets does not occur in utero. Until further studies of the bones of new-born children are made, it is probably unsafe to conclude that rickets can never develop in intra-uterine life. One may be certain, however, that if rickets ever occurs in intra-uterine life it does so rarely, and it never exceeds the slightest degree of development.

*The earliest age at which rickets occurs.* It is commonly taught that rickets first begins about the third or fourth month, because the first clinical signs of the disease, head sweating and craniotabes, make their appearance about then. When, however, clinical evidences of the disease exist, the pathological changes in the bones have become fairly advanced, and the disturbance in metabolism which gives rise to them must have

been in existence for some time. Anatomical studies of large numbers of children, from the time of birth to four years of age, have been made by Schmorl (6) with a view to determining the age incidence of rickets. The earliest age at which Schmorl was able to find evidence of the disease was one and one-half months. In this clinic, however, Dr. Ethel Dunham<sup>2</sup> has discovered well-advanced rickets in a prematurely born infant aged only one month. Both Yllpö (16) and Hamilton (17) have commented on the fact that rickets makes its appearance earlier in prematurely born infants than in those born at term.

If rickets can become sufficiently advanced to manifest itself at the age of one month in lesions which are easily demonstrable by the x-ray at the ends of the long bones of the extremities, it is clear that the disease commenced immediately after birth. Doubtless rickets actually begins in the case of many children immediately after birth. Indeed, such cases as the one discovered by Doctor Dunham make one hesitate to think that rickets cannot begin in intra-uterine life.

*The frequency in prematurely-born infants.* Both rickets and tetany are exceedingly prevalent in infants born before term. According to Rosenstern (18), 76 per cent of all premature infants exhibit evidences of tetany. The statistics of Yllpö (16) indicate that 35 per cent of premature infants have tetany. As is obvious, no one can say how frequently rickets occurs in infants whether prematurely born or at term. It is certain, however, that the great majority of premature infants, in the countries in which rickets is common, develop the disease whether suckled or fed artificially.

It is interesting that both tetany and rickets are apt to manifest themselves differently in premature infants than in those born at term. The tetany appears early, is apt to be latent and becomes manifest only in the presence of infection. It tends to disappear naturally about the sixth month if breast milk feeding is continued. The rickets shows itself early, as has already been pointed out, and is characterized by deformities of the head which are out of proportion to those of the extremities, a fact of general knowledge emphasized recently by Hutchinson (19) and Yllpö (16).

*The seasonal variation.* The observation that there is a seasonal variation in the incidence of rickets and tetany was made years ago and has been remade many times since.

In 1884 Kassowitz (20) commented at length on the rise in the curves of incidence of rickets and tetany during the winter months and their

<sup>2</sup> The report of this case is to be published.

decline during the summer and autumn, and he used the parallelism between them as an argument for the unity of tetany and rickets.

The seasonal variation in rickets was beautifully demonstrated in the pathological studies of Schmorl (6). These studies showed that rickets may begin at any time, but the highest percentage of early manifestations of the disease is between November and May. The percentage of cases with signs of healing increased as the summer progressed and reached its highest point in the autumn, only to fall again as the winter months came.

Hansemann (11) noted that almost all children who are born in the fall and die in the spring show marked manifestations of rickets, in contrast to the children born in the spring and dying in the fall who are almost free from rickets.

In this country Hess (21) has emphasized the importance of the seasonal variation in rickets, and recently Lundagen and he (22) have reported a seasonal variation in the level of the inorganic phosphate of the blood (270 determinations; the number of children investigated not stated). The average figures for the inorganic phosphate of the blood serum were: June and July, 1921, 4.35 mgm. per cent; December, 3.92 mgm.; January, 1922, 3.9 mgm.; February, 3.76 mgm.; March, 3.58 mgm.; April, 3.68 mgm.; May, 3.98 mgm. The authors conclude that there is a seasonal variation in the level of the inorganic phosphate of the blood of the normal infant which corresponds to and is determined by the seasonal variation in the richness of the solar spectrum in ultra-violet rays. Of course, in order to be sure that a seasonal variation in the blood phosphate is a normal phenomenon in the infant, it would be necessary to examine repeatedly during a year great numbers of infants and children living at widely separated places and under the most varied conditions. Unfortunately, in the paper by Hess and Lundagen data on this point are lacking. It seems most improbable, however, that slight seasonal differences in the level of the inorganic phosphate of the blood found by Hess and Lundagen are to be interpreted as meaning that a "seasonal tide of the blood phosphate" is a physiological phenomenon; but rather that at the time of the year when the incidence of rickets is highest the disease in its mildest form is almost universal under the conditions of hygiene and diet which surround infants in cities, in particular those living in institutions. The infants studied by Hess and Lundagen were probably living under conditions commonly accepted as normal for the infant but were not those which nature intended. We know already, from the

work of Schmorl and others, how extraordinarily widespread rickets is among the infants and children of cities. Schmorl (6) found that 90 per cent of the children in Dresden under four years of age who died between the years 1901 and 1908 and more than 96 per cent of the infants showed evidences of rickets, and Hess (23) himself has reported that rickets was exceedingly prevalent among the children in the Hebrew Infant Asylum in New York City, where presumably the chemical investigations of the blood were made. The investigations of Hess and Lundagen, then, are probably to be regarded as further corroborations of the seasonal variation of rickets and of the universality of rickets among infants and children living under the conditions which prevail in our large cities.

*The association with other diseases.* Rickets frequently accompanies infantile scurvy; indeed, it is exceedingly uncommon to find scurvy uncomplicated by rickets. The association of rickets and tetany is also exceedingly close. A very large proportion of children with rickets show also tetany, as has been pointed out by Kassowitz (20) and many others, and recently by Ferguson (24).

THEORIES CONCERNING THE CAUSE OF RICKETS: *Several hypotheses that need only be mentioned.* Glisson (2) believed that rickets was the result of over-eating (over-nutrition) (8) and recently Jundell (25) has revived this idea. Heitzmann (26) proposed that the disease was caused by an acidosis which brought about a decalcification of the bones, a hypothesis which has been advanced recently by Klose and Vogt (27) in explanation of the rickets which their thymectomized dogs developed and which has been advocated also by Pritchard (28). Several French clinicians (29) have held that rickets is a manifestation of congenital syphilis or have attributed it to auto-intoxication. The theories that rickets is the result of infection or is inherited or is due to the disturbance in the functions of the organs of internal secretion, will be considered in that portion of the paper which immediately follows. The two most important theories, namely, that rickets is due to deprivation of light or is caused by defective diets, will be discussed at length in the remainder of the paper.

*Is rickets inherited?* Siebert (30) is the exponent of the theory of inheritance. He made statistical studies of thirty-one families in which the children were breast-fed but had rickets, and of fourteen families in which the children were breast-fed but were free from rickets. In twenty-nine of the families of the first group he found that the mother either had deformities of rickets or gave a history of rickets, and in the remaining

two families he discovered evidence which made the previous existence of rickets in the mother probable. In the second group, in which the children were free from the disease, the parents also were free. When he investigated families in which the rachitic children were artificially fed, the incidence of rickets in the parents was not so striking. In the main, however, Siebert found that one or both parents had been sufferers from rickets if the children had the disease. Siebert concluded that heredity is the most important factor in the etiology of rickets and that the disease is most commonly transmitted through the mother, though he brought forward some evidence to indicate that it might be transmitted through the father. If both parents were rachitic, he thought rickets in the offspring was inevitable. Siebert was greatly puzzled on finding some children in the rachitic families who had escaped the disease. It is an amusing sidelight on the subconscious workings of the mind of an investigator over-intent on his theory, that Siebert should have thought such children were probably illegitimate. The family history of rickets might be taken as supporting the theory that heredity bears a part in its cause. It might also be taken as evidence that rickets is of the nature of an infection, or it may simply mean that the habits and ways of living of one generation have been continued in the next. Inasmuch as rickets consists in a disturbance of metabolism, however, a predisposition may be inherited. Pigmented skins apparently increase the susceptibility to rickets, (31) and a predisposition in that sense may be inherited. The disease, however, cannot be inherited through the germ plasma.

*Is rickets the result of a disturbance in the function of the organs of internal secretion?* Rickets has been ascribed to disturbances in the function of the thyroid, parathyroid, thymus and adrenal glands, and the carotid bodies. Lanz (32) suggested the use of the thyroid gland in rickets, but Knoepfelmacher (33) and Heubner (34) tried it without success. Stoeltzner (35) announced favorable results from the use of the adrenal gland, but other investigators were unable to corroborate his findings, and finally even Stoeltzner is said to have abandoned the idea (30). A large number of investigators have reported that the removal of the thymus caused rickets. Chief among them may be mentioned v. Basch (36), Klose and Vogt (27) and Matti (37). Their work has been challenged and overturned, however, by Nordmann (38), Pappenheimer (39), Renton and Robertson (40) and McClure and the writer (41) of this paper. Erdheim (42) has reported that rickets follows removal of the parathyroid glands. Both Erdheim (42) and

Pappenheimer (43) have found the parathyroid gland to be hypertrophied in rickets. It is probable, however, but not certain that the function of the parathyroid glands has no causative relationship to the development of rickets. The *reductio ad absurdum* of the theories of interrelationship between the function of the organs of internal secretion and the development of rickets is to be found in the work of Betke (44). This investigator reported that removal of the carotid bodies was followed by the development of rickets and the same elaborate train of symptoms which Klose and Vogt described after removal of the thymus.

While it is conceivable that the vitaminines or radiant energy are connected in some way with the functioning power of the organs of internal secretion, it seems most probable that the rickets which developed in animals after removal of the organs of internal secretion was due to other causes. Pappenheimer encountered rickets both in his thymectomized rats and in his control rats. Similar experiences are not at all uncommon in the case of animals in laboratories.

*Is rickets a result of infection?* Iovane and Forte (45) and Moussu (46) advocated the infectious origin of rickets. Their experiments, however, had little value.

Morpurgo (47) in 1898 noted the spontaneous appearance of a disease resembling rickets and osteomalacia in a colony of white rats of stock which had been in the laboratory for seven years. He obtained a diplococcus from the infected animals, inoculated other animals with it, and found that they developed the disease. Morpurgo supplies no information concerning the food which the rats received. The organism was not recovered from any of the inoculated rats.

Koch (48) also reported the production of rickets by means of inoculation with bacteria. Altogether Koch inoculated seventy dogs, and had more than one hundred control dogs. After preliminary experiments he chose the streptococcus longus as the organism most likely to produce rickets. During the first three or four days the dogs developed swollen joints and showed symptoms which were evidently the result of infection. In typical instances a period then followed during which the animals seemed well. Before long, however, rachitic deformities appeared and reached an advanced development. The dogs were kept in horse stalls and were fed milk, semmel, meat and potatoes, with the addition of salt. Koch states that three of thirty control dogs also developed rickets. Koch interpreted his experiments as meaning that rickets is produced by an organism having a special

affinity for the skeleton. The arthritis and constitutional symptoms marked the invasion of the skeleton, and represented the acute stage of the disease; the rachitic deformities were the results of the inflammation after it had become chronic.

Years ago Edlfsen (49) favored the infectious origin of rickets as the result of observations on children. He thought the enlargement of the spleen in rickets significant and regarded fever as an early manifestation. The recurrence of rickets in certain houses suggested that the houses harbored some infectious agent.

A careful search of the literature would bring to light the names of many exponents of the infectious origin of rickets. Clinical experience and investigation, however, have made clear that infection cannot possibly be more than a contributory factor.

THE EXPERIMENTAL PRODUCTION OF RICKETS<sup>3</sup> IN ANIMALS AND THE KNOWLEDGE DERIVED THEREFROM. Sixteen years ago Hopkins (50) wrote: "But, further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet. Lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored; only is it certain that there are many minor factors in all diets, of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure" (p. 395). In the book entitled "Die Vitamine" by Funk (51), published in 1914, the following sentence appears: "It is very probable that rickets occurs only when certain substances in the diet essential for normal metabolism are lacking or supplied in

<sup>3</sup> Voit, Diblett, Aron and Sebauner, and Stolzner and Miwa have attempted to produce rickets in animals through the administration of diets deficient in calcium. Their experiments took place before the importance of an exact understanding of the composition of the diet was appreciated, and before the pathology of rickets had become generally understood. Although their work contains much information of great value, no presentation of it will be made in this paper.

insufficient amount. The substances occur in good breast-milk, also in cod liver oil, but are lacking in sterilized milk and in cereals" (p. 127). Funk had reference to the so-called "vitamines." When, accordingly, Mellanby (52) made the announcement in 1918 of the successful production of rickets by means of a diet lacking in "an accessory factor," medical opinion was in a measure prepared. The report of his first experiments was meagre and would probably have awakened little interest, had not the British Medical Research Committee endorsed the work and publicly committed itself to the view that rickets is a deficiency disease due to a lack in the diet of an "anti-rachitic factor" (53).

Although the experiments of Findlay (54) preceded those of Mellanby by ten years, it was the work of Mellanby which gave to the investigation of rickets its impetus. For this reason and also because the more recent experiments of Findlay, Paton and their co-workers seem to have been designed to attack the dietetic theory of rickets, the experiments of Mellanby will be described before those of the Glasgow School.

*The experiments of Mellanby.* Mellanby's experiments (55) were performed entirely on puppies and reached a total of almost four hundred. The animals were about two months old when the experiments were begun, and were kept on the test diets for a number of weeks or months until rickets had developed or had failed to develop. The diet used by Mellanby as a standard for the production of rickets in the puppies underwent a natural evolution as may be seen from the following table:

*Rachitic diets*

DIET I	DIET II	DIET III	DIET IV
Whole milk, 175 cc.	Whole milk, 175 cc.	Separated milk, 175 cc.	Separated milk, 250-350 cc.
Oatmeal, rice.	Bread ad lib.	Bread (70 per cent wheaten) ad lib.	Bread (70 per cent wheaten) ad lib.
1-2 gm. NaCl.		Linseed oil, 10 cc. Yeast, 10 gm. NaCl, 1-2 gm.	Linseed oil, 5-15 cc. Yeast, 5-10 gm. Orange juice, 3 cc. NaCl, 1-2 gm.

The principle on which Mellanby proceeded was as follows: Having established a standard diet which regularly produced rickets in the puppy, he introduced into it in turn food substances whose influence

for or against rickets he desired to determine. For example, in experiment 282 he substituted 10 grams of lard for the linseed oil. The development of rickets in the puppy was uninfluenced. Mellanby was then able to infer that lard contained no substance of an anti-rachitic nature. The great majority of Mellanby's dogs were confined indoors. In a few experiments, however, in particular in some of those in which the influence of confinement on the development of rickets was under investigation, the animals were either confined out-of-doors or allowed a run in an enclosure. One assumes that the majority of his dogs lived in roomlight (daylight filtered through ordinary window glass). There is every reason to suppose that his animals received excellent care. The dogs under observation seem to have been abundantly controlled, a point of great importance, though the control animals were not from the same litters.

The criteria which Mellanby used to determine whether a puppy had or had not rickets were: *a*, the appearance; *b*, the x-ray findings; *c*, the determination of the calcium oxide in the bones; and *d*, histological study of the bones. The reader gains the impression that Mellanby himself had no special knowledge of the histological changes characteristic of rickets, and that he depended largely on x-ray examination and on the determination of calcium oxide.

Some very interesting results of Mellanby's experiments are the following: Among the oils, cod liver oil was found to stand alone in its anti-rachitic potency; suet and butter fat exerted a strong influence on calcification, while lard had no anti-rachitic effect; of the vegetable oils, peanut oil stood first, then cocoanut oil; rapeseed, cottonseed, palm kernel, olive and linseed oils produced little or no effect; babassu oil was the "worst;" cod liver oil was found to be superior to butter fat and invariably prevented rickets; butter fat did not always prevent rickets. Mellanby (55) reached the very interesting conclusion that "butter is a more potent anti-rachitic agent when it has abundance of calcium salts, as found in milk, with which to work. It is but natural that the anti-rachitic vitamin of butter, which certainly has a strong influence on the deposition of calcium phosphate in bone, should also have a sufficiency of calcium salts in the diet before it can work effectively" (p. 53).

Mellanby performed a most interesting experiment which has since been repeated with much greater success by McCollum, Simmonds, Becker and Shipley (56). Hopkins (57) had shown that when the temperature of butter fat or cod liver oil is maintained at 120°C. for four

hours in a stream of oxygen, fat-soluble A is destroyed. Mellanby fed butter and cod liver oil, oxidized in this way by Hopkins himself, to puppies on the standard diet. The puppy receiving from 5 to 10 grams of oxidized butter was not protected from rickets, whereas the puppy receiving the untreated butter was protected. In contrast, the puppy receiving 10 cc. of oxidized cod liver oil was completely protected from rickets. If Mellanby had made certain that the oxidized cod liver oil was devoid of fat-soluble A, he would have known that the substance in cod liver oil which prevented rickets was not fat-soluble A.

Mellanby found that lean meat has an anti-rachitic action which seemed, however, to be of a different order from that of the vitamine-containing fats. Meat, he observed, made the puppies eat better and, under favorable experimental conditions, prevented or limited the extent of the rickets; under unfavorable conditions it did not prevent the development of rickets. Bread seemed to favor the development of rickets. The more bread eaten, the more marked the rickets. Casein retaining its calcium (alkaline casein) prevented the development of rickets; casein from which the calcium had been removed by treatment with hydrochloric acid (acid casein) seemed to intensify the rickets-producing power of the diet. The feeding of thyroid gland did not inhibit the development of rickets. Some animals, in particular those receiving the purified casein, showed signs of tetany.

Some of Mellanby's general observations are interesting. If the diet was well constituted, a small amount of the "anti-rachitic vitamine" appeared to suffice; if badly constituted, in a rachitic sense, a large amount was necessary. The older the animal, the more difficult the production of rickets and the more independent of the "anti-rachitic vitamine" the animal seemed to be. Mellanby observed that "osteoporosis" in dogs may give rise to deformities identical with those of rickets. The addition of tricalcium phosphate to the standard diet did not prevent rickets.

The view of Mellanby concerning the causation of rickets was broad. Though holding rickets to be a deficiency disease due to the absence of an "anti-rachitic vitamine" in the diet, he believed that defective environmental conditions and confinement contributed to its development.

The experiments of Mellanby were open to obvious criticism: his diets were more crude than those of some other investigators; the salt composition of the diets was not taken into consideration; good histolog-

ical studies of the bones and chemical examinations of the blood were lacking; the criteria on which chief reliance was placed were not satisfactory. Nevertheless, the experiments of Mellanby constitute pioneer work and as such were splendid. To him belongs the credit of establishing the relationship between the development of rickets and the deficiency in the diet of a vitamine.

*Experiments of Findlay and the Glasgow school of investigators.* In 1908 Findlay (54) published his well-known experiments on the production of rickets by confinement. Led by the idea that rickets was a dietetic disorder, he first attempted to produce it by feeding diets composed of cereals or bread and water, but found that the puppies became marantic and did not develop rickets. Control puppies, however, fed a diet of milk and porridge, did develop rickets, with the exception of one animal which, as it happened, was exercised daily in the open air. In this way Findlay's attention became attracted to the possibility of a connection between rickets and muscular activity. Findlay renewed his experiments. The test animals were kept in cages in the laboratory, while the control animals were allowed to run about the floor. The diet was milk and porridge. Eight confined puppies developed the disease, whereas the unconfined puppies remained free. Findlay stated with great positiveness that rickets ". . . . is due to the want of exercise which invariably goes along with it or is consequent on confinement" and concluded with the sentence: "Alike, then, on clinical and experimental grounds, I accordingly conclude that confinement, with consequent lack of exercise, is the main factor in causing the disease."

The diet used by Findlay was poor and similar to the one employed subsequently by Mellanby for the production of rickets in puppies. Good control experiments amounted only to five. No doubt exists that Findlay's puppies had rickets.

The stir created by Mellanby's work had already become apparent, for in 1918 Paton, Findlay and Watson (58) reported experiments which seem to have been planned to put Mellanby's theories and theirs to a comparative test; and in 1921 Paton and Watson (59) made an actual foray into Mellanby's domain. Space does not permit a description of these interesting but rather complicated experiments of the Glasgow investigators. It is sufficient to say that the experiments indicated: dogs allowed a run in the country do not develop rickets; butter fat does not prevent rickets, but possibly exerts a slight protective action; high calorie diets rich in whole milk seem to exercise some protective influence; confinement alone does not cause rickets.

*Rickets cannot be due to "confinement with lack of muscular exercise."* The idea that the primary cause of rickets lies in the inability of the child to gratify a natural impulse for exercise is difficult to accept on *a priori* grounds. Some years ago Howland and the writer<sup>4</sup> of this paper, and, later, Baldwin<sup>4</sup> confined puppies in small cages for two or three months, but could not obtain rickets in that way. Mellanby also showed clearly that confined puppies will not develop rickets if the diet is properly constituted. Though Paton and Watson failed to emphasize the fact that their experiments did not turn out in accordance with the theory of confinement, both they and Findlay must have been conscious of the fact, for in 1921 Paton showed signs of retiring upon the hypothesis that rickets is due to infection (59), and Findlay has recently withdrawn to a new position where nature has erected an unassailable stronghold,—sunlight. In his most recent paper on the subject of rickets Findlay (60) has written: ". . . defective hygiene, using this term in its widest sense, is the most important known determining factor in the causation of rickets" (p. 826).

*The experiments of American investigators.* Almost simultaneously Sherman and Pappenheimer and McCollum, Simmonds, Shipley and the present writer published papers reporting the production of rickets in the rat by means of diets having specific defects in their salt composition.

In January, 1921, McCollum, Simmonds, Shipley and the present writer (61) reported that a number of different diets produced abnormal conditions in the skeleton of the rat which corresponded closely to or appeared actually to be identical with the rickets of the human being. All of these diets were known to be defective in fat-soluble A. One was defective in its phosphorus content, though the significance of this was not appreciated at the time; the others were defective in calcium. In general the proteins used were of poor quality. The investigators had before them at that time what subsequent investigation revealed to be two kinds of rickets, one characterized by a calcium phosphorus ratio in the blood which was low, the other by one which was high. Conditions appeared to be too complicated to permit of any single explanation. The writers, therefore, stated: "At present it is only possible to say that the etiological factor is to be found in an improper dietetic regimen. The large number of dietary formulae, the administration of which results in rickets and kindred affections,

<sup>4</sup> These experiments were never reported.

gives abundant evidence of the complex nature of the causes operating in the production of the disease— . . . ." (p. 340).

In March, 1921, Sherman and Pappenheimer (62) published a most important paper which described the production of rickets in the rat by means of a diet low in phosphorus and its prevention by means of the addition to the diet of alkaline potassium phosphate. The diet was composed of patent flour, 95 per cent, calcium lactate, 3 per cent, sodium chloride, 2 per cent, with or without the addition or a trace of ferrie citrate; this regularly produced rickets which possessed all the characteristics of the disease in the human being. When alkaline potassium phosphate was added to the diet in the proportion of 0.4 per cent, and an equivalent amount of calcium lactate was withdrawn, the animals were completely protected from the development of rickets. The diet which Sherman and Pappenheimer used was deficient not only in fat-soluble A and phosphate but in water-soluble B, water-soluble C, protein and potassium. Accordingly, it was impossible to be certain that the rickets occurred solely as the result of the deficiency in the phosphorus. In publications which soon appeared (63), (64), (65), however, Pappenheimer, McCann, Zucker and Hess showed that the additions to the diet of yeast in small amounts, orange juice, butter fat in moderate amount, and potassium did not exert the inhibitory influence.

The paper of Sherman and Pappenheimer was soon followed (May) by the publication of kindred experiments by Shipley, McCollum, Simmonds and the present writer (66). These investigators reported the production of rickets by means of two diets. One was composed of rolled oats 40 per cent, flaxseed meal 8.3 per cent, sodium chloride 1.0 per cent, calcium carbonate 1.4 per cent, dextrin 49.2 per cent. The other was composed of rolled oats 40.0 per cent, gelatin 10.0 per cent, sodium chloride 1.0 per cent, calcium carbonate 1.5 per cent, and dextrin 47.5 per cent. Both of these diets were known to be deficient only in fat-soluble A and in phosphorus, and in other respects were well constituted. When a complete salt mixture was substituted for the inorganic constituents of diet 2806, so that the deficiency in phosphorus was removed, the diet no longer produced rickets, but typical osteoporosis.

The experiments of Sherman and Pappenheimer and McCollum, Simmonds, Shipley and the present writer marked a distinct advance in the investigation of rickets in several respects, especially because they showed the importance of the inorganic constitution of the

diet in particular of the phosphorus, in relation to the disease. With the exception of Mellanby, who had placed but little importance on the salt composition of the diets, other investigators had attempted to produce rickets by means of reduction of the calcium.

Since the subsequent experiments of Pappenheimer and those associated with him, Hess, Zucker, McCann and Steiner, and the experiments of McCollum, Simmonds, Shipley and the present writer showed a remarkable parallelism and led to almost identical conclusions, they will not be treated separately.

*Cod liver oil cures rickets and prevents its development.* According to Trousseau, cod liver oil has been used from time immemorial as a folk-remedy on the coast of England, Holland and France. It was introduced into France as a specific for rickets by Bretonneau, and its uses became general through the teachings of Trousseau. Trousseau himself was convinced of its curative action not only in rickets but also in osteomalacia and many physicians have been certain of its effectiveness in rickets solely as the result of clinical observation. Schabad (128), Orgler (122) and Freund (130) have brought forward indirect evidence of the curative action of cod liver oil in rickets by means of metabolism experiments which will be referred to later. The direct proof of its curative action, however, was first obtained by McCollum, Simmonds, Shipley and the present writer (74). These investigators discovered early that cod liver oil caused remarkable depositions of lime salts to form in the cartilage of the rachitic rat close to its junction with the metaphysis (the so-called "line test"). Howland and the present writer (139) proved by means of the x-ray that the administration of cod liver oil to rachitic children was followed by the deposition of lime salts in the cartilage and bones after a period of fifteen to twenty-one days.

*The relationship of fat-soluble A to the development of rickets.* Diets deficient in fat-soluble A alone do not produce rickets. This fact was first clearly proved in the experiments of Sherman and Pappenheimer (62) and in those of Shipley, McCollum, Simmonds and the present writer (66) already described. The group of investigators last mentioned clearly stated that "a deficiency in fat-soluble A cannot be the sole cause of rickets," (p. 165) and that the pathological condition induced by diets deficient in fat-soluble A alone was "osteoporosis." The subsequent experiments of Pappenheimer and the investigators associated with him (67) and of McCollum, Simmonds, Shipley and the present writer (68) have repeatedly attested to the truth of these observations. Zilva, Golding, Drummond and Coward (69) Mackay (70) and Tozer (71)

also came to similar conclusions as the result of experiments on the pig, kitten and guinea pig. There can be no doubt that in man a deficiency in the diet of fat-soluble A alone does not give rise to rickets. Indeed, there is evidence to show that fat-soluble A in the diet may be necessary for the full development of the rachitic process, as will be pointed out later.

*The organic substance or factor in cod liver oil which causes lime salts to deposit in the bones in rickets is in all probability distinct from fat-soluble A.* (In order to avoid constant repetition, it will hereafter be termed X. By X is understood that substance or group of substances, factor or factors so richly contained in cod liver oil, which exert the regulatory influence on the metabolism in rickets and bring about the deposition of lime salts in the bones.)

Believers in the dietetic origin of rickets were in a dilemma. The investigators working with rats had shown that rickets could be made to develop by means of diets defective in their salt composition, but not if at the same time the animal was receiving cod liver oil. Cod liver oil obviously contained some substance inhibitory to the development of rickets. Yet in the absence of cod liver oil from the diet, rickets would not result if the salt defect was removed by the addition of phosphate or by the substitution of a well-balanced salt mixture. Obviously the development of rickets in the rat depended, other things being favorable, not on a single contingency but on two contingencies: *a*, the absence from the diet of an organic substance contained in cod liver oil which, so to speak, threw open the door; and *b*, a disproportionate relation in the inorganic constituents of the diet which, so to speak, caused rickets to enter. Now cod liver oil was especially rich in fat-soluble A. The natural assumption was, therefore, that the inhibitory organic substance in cod liver oil was fat-soluble A. Butter fat, however, also rich in fat-soluble A, exerted an exceedingly feeble action. No advocate of the dietetic theory of rickets could deny, on the one hand, that an organic factor was concerned in the development of the disease, or, on the other hand, dared say that an organic factor distinct from fat-soluble A was concerned.

In July, 1921, McCollum, Simmonds, Shipley, and the present writer, influenced by the differences in the effectiveness of butter fat and cod liver oil when added to the diets of rats in a state of calcium starvation, suggested the existence of a dietary essential in cod liver oil separate from fat-soluble A (72). As the result of further experiments on the rat with diets containing butter fat and cod liver oil, the investigators just

named were able to write, in January, 1922, the following statement (73): "The results of this series of experiments were so consistent and decisive that we can deduce no other conclusion than that cod liver oil contains in abundance some substance which is present in butter fat in but very slight amounts, and which exerts a direct influence on the bone development, and enables animals to develop with an inadequate supply of calcium much better than they could otherwise do. This substance is apparently distinct from fat-soluble A, which is essential for growth and which is associated definitely with the prevention of ophthalmia (keratomalacia)." (p. 7.)

In June, 1922, McCollum, Simmonds, Becker and Shipley (56) succeeded in obtaining striking evidence of the existence of a substance in cod liver oil distinct from butter fat which causes the deposition of lime salts in the bones of rats rendered rachitic by the diet. The proof depended on the use of the method developed by Hopkins (57) for the destruction of fat-soluble A in oils already described in connection with Mellanby's experiments, and of the so-called "line test" by which the power of any food substance to cause the deposition of lime salts in the bones could be determined. McCollum, Simmonds, Shipley and the present writer at the very outset of their work had demonstrated that the sudden introduction of cod liver oil into the diet of a rachitic rat is followed by a beautiful deposition of lime salts in a transverse line across the cartilage (74). This phenomenon was utilized for the development of a delicate biological test for lime salt-depositing substances. The four investigators previously mentioned first destroyed fat-soluble A in cod liver oil according to the method of Hopkins. They, then, having proved the destruction of fat-soluble A by demonstrating the ineffectiveness of the cod liver oil for the cure of xerophthalmia, fed it to rats rendered rachitic by means of the diet and discovered that its power to produce deposition of lime salts was retained.

It is difficult to escape the conclusion that a factor or factors exist in cod liver oil distinct from fat-soluble A which cause lime salts to deposit in rachitic bones.

*X appears to be closely associated with and related to fat-soluble A.* A reason which made Mellanby think the "anti-rachitic dietary factor" identical with fat-soluble A was that the power possessed by various fats to prevent the development of rickets, as demonstrated in his experiments, goes hand in hand with their known content of fat-soluble A, as shown by others. In other words, fats known to contain fat-soluble A appeared also to possess the property of preventing, at least

in some measure, the development of rickets. A second reason which inclined Mellanby to favor the identity of the "anti-rachitic vitamine and fat-soluble A" was that the dependence of the animal on fat-soluble A, on the one hand, and on the unknown "anti-rachitic factor," on the other, varies directly with the age. As animals grew older, they tended to become less dependent on both. Of course, the correspondence between the content of fat-soluble A in certain fats and their power to cause lime salts to deposit in the bones of the subjects of rickets does not seem to be so close as Mellanby supposed. McCollum, Simmonds, Becker and Shipley (56) have presented evidence to show that cocoanut oil lacks the power to cure xerophthalmia, but has the power to cause the deposition of lime salts in the rachitic skeleton. Butter fat seems to possess a power to prevent or cure xerophthalmia in excess of its power to prevent or cure rickets, as will be discussed more fully below. In general, however, as far as our limited knowledge extends, it can be said that fats containing fat-soluble A tend to contain also X. The greatest argument, however, for thinking that the fat-soluble A and the substance operative in rickets might be the same is to be found in the results of a splendid investigation by Zucker, Johnson, and Barnett (75). These investigators hydrolyzed cod liver oil with sodium hydroxide and separated the fatty acids. The fatty acids were found to be entirely inactive when put to the test on the rachitic rat. The residue, however, exerted a marked curative action. The bases were then removed from the residue and shown to be inactive. The cholesterol fraction was next crystallized from the residue and found inactive. Finally, a fraction was left which gave a curative effect a little stronger than the original oil when diluted with ninety parts of cottonseed oil. This fraction had retained its power to cure xerophthalmia. If Mellanby and the committee of the Medical Research Council which supported him can be accused of "barking up the wrong tree," they at least deserve the great credit for having "barked up a tree" which stood close by the right one. As a matter of fact, Mellanby never stated that the "anti-rachitic vitamine" and fat-soluble A were identical, but that *most probably* they were identical.

*The distribution of X in foods.* Knowledge concerning the distribution of X in foods is limited. X is present in large amounts in the oil of the cod and in a considerable amount in the oils of the shark and burbot (a fresh water fish) (56), (76). It is present in small amount, as compared with cod liver oil, in butter fat. Cocoanut oil contains it. Other vegetable oils studied, among which were olive oil, maize oil and linseed oil, appear to be entirely free from it.

*Is X to be regarded as a normal constituent of foods?* Zucker, Johnson and Barnett (75) state that rickets cannot be ascribed to a "vitamine deficiency" until the curative agent in cod liver oil has been shown to be a normal component of foods. The fish oils, butter fat, and cocoanut oil are foods. It is already necessary to regard X as a component of foods. The only uncertainty relates to the extent of its distribution in foods and its concentration in those foods in which it occurs. Probably X, like fat-soluble A, occurs abundantly in green leaves. There is urgent need of further investigation of this subject.

*The influence of radiant energy<sup>5</sup> in the prevention and cure of rickets.* As already said, many observers have noted the tendency of rickets to appear in children who lived in the crowded, dark, ill-ventilated rooms of city dwellings. Most of these observers, however, became fixed by the idea that bad influences were there at work, such as "noxious gases," "infection," and failed entirely to consider the alternative of a powerful preventive and curative force in sunlight. A few did perceive the truth. In a remarkable paper on the etiology of rickets (1890), Palm (88) recognized the full importance of sunlight and gave remarkable recommendations for the eradication of the disease by means of sunlight. Raczynski wrote (89) (1912) "that it is the sun which plays the principal rôle in the etiology of rickets," and gave the first proof of the favorable influence of light on mineral metabolism by an experiment on puppies. One puppy was reared in the dark, the other in the sunlight; both were suckled by the mother. At the end of six weeks Raczynski found the calcium oxide and the phosphorus pentoxide in the body of the puppy reared in sunlight to be greatly in excess of that found in the body of the puppy reared in darkness. Raczynski concluded with this statement: "It is possible to assume that the lack of action of sunlight by influencing in so unfavorable a manner the assimilation of calcium oxide in the young organism is one of the causes of rickets. This is in complete accord with clinical experience." The favorable influence of sunlight in the treatment of rickets has been emphasized by Feer (91), and Neve (90).

Almost twenty years ago Buchholz (92) attempted to treat children with rickets by means of light from artificial sources. He used the "gluhlicht"<sup>6</sup> and reported favorable results in sixteen cases. It is

<sup>5</sup> By radiant energy is meant those radiations chiefly, if not entirely, emitted from the ultraviolet portion of the spectrum which exert the remarkable influence on the metabolism in rickets. The term *light* is avoided because some, if not all, these radiations are invisible.

<sup>6</sup> The carbon filament electric light bulb.

possible that the credit for the discovery of the curative action of light in rickets belongs to him, but, since accurate information concerning his work is lacking, it is necessary to assign this great discovery to Huldchinsky.

In June, 1919, Huldchinsky (93) reported that the ultra-violet ray exerted a curative action in rickets. He had treated four children who had advanced rickets with the mercury vapor quartz lamp, and found that at the end of four weeks it was possible to demonstrate with the X-ray deposits of lime salts at the ends of the long bones of the extremities. After two months the healing seemed almost complete.

In September, 1919, Winkler (94) reported the cure of rickets by means of the X-ray. He used a medium soft tube at a focal distance of about 20 cm. The exposure did not exceed ninety seconds and was repeated every other day. The treatment which was directed against the craniotabetic lesions of the head resulted in the gradual disappearance of all manifestations of the disease. Winkler states that he was able to demonstrate the deposition of lime salts at the ends of the radius and tibia by means of X-ray photographs. Hess, Unger and Steiner (95) have been unable to corroborate Winkler's work.

The discovery by Huldchinsky of the curative action of light in human rickets has been corroborated by Putzig, (96), Karger, (97) Huldchinsky himself (98) in numerous additional experiments, Riedel (99), Sachs (100), Erlacher (101), Mengert (102), Hess and Unger (103), Kramer, Casparis and Howland (104) Chick and her collaborators (105), and many others.

Huldchinsky found that the action of light in causing lime salts to deposit in the bones was general, not local. By raying one arm of a rachitic child, lime salts were made to deposit in the other.

Sachs (100) reported that treatment with the ultra-violet ray cured latent tetany and at almost the same time Huldchinsky (106), and a little later Sachs (100) himself, reported cures of manifest tetany by that means.

Huldchinsky (98), made use of sunlight in combination with the mercury vapor quartz lamp in the treatment of two rachitic children; in some cases Riedel (99) relied mainly on treatment with sunlight and used artificial light only when sunlight was unavailable. Hess and Unger (107), however, first demonstrated that sunlight alone possesses the curative action exercised by the light of the mercury vapor quartz lamp in the rickets of human beings. Hess, Unger and Pappenheimer (108) and Powers, Shipley, McCollum, Simmonds and the present writer

(109) investigated the influence of sunlight in the prevention of rickets in the rat and found the prevention to be complete. When the rats were kept out-of-doors in the sunlight, diets, which regularly produced rickets indoors in a medium of roomlight, failed entirely to produce the disease. Later these two groups of investigators (110), (111), (112) further corroborated Huldchinsky's findings by showing that the mercury vapor quartz lamp protects the rat against the development of rickets. Hess, with several collaborators (110), Shipley (140), and Howland and Kramer (76) have reported the protective action of light from other artificial sources known to yield the ultra-violet ray (the carbon arc light, the cadmium spark and the ferric chromium condenser). It is interesting that the tungsten arc which gives a rich line spectrum, extending as far as  $210 \mu\mu$ , does not protect rats from the development of rickets.<sup>7</sup>

*The radiations which are effective in rickets.* The spectrum of the sun is continuous, ranging from the long infra-red to short waves

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#### ERRATUM

*Volume III, No. 1 (January, 1923):* Page 127, line 12, instead of "tungsten arc" read "tungsten filament."

duce photochemical reactions lie at the ultra-violet end of the spectrum.

Hess, Pappenheimer and Weinstock (113) have shown that in the case of the rat the rays of light which protect against rickets lie in the ultra-violet zone and are about  $300 \mu\mu$  in length, or shorter. The question exactly which are and which are not the active rays, is complex, since two factors must be considered—the length of the rays and their intensity. It is possible, for example, that rays of light which exert only a feeble effect in the cure of rickets may have great effect if supplied in great intensity as in sunlight. While the general conclusion of Hess, Pappenheimer and Weinstock is not to be doubted, large numbers of experiments must be made with both variables taken into consideration before it will be possible to consider this question as settled.

<sup>7</sup> Results of experiments by Dr. H. Laurens and Dr. G. F. Powers which have never been reported.

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*The radiations which are effective in rickets.* The spectrum of the sun is continuous, ranging from the long infra-red to short waves about  $290\ \mu\mu$  in length. The mercury vapor quartz lamp has a discontinuous spectrum emitting a number of bright lines, from  $595\mu\mu$  to about  $240\mu\mu$ , below which there are a number of weak lines. All investigators, beginning with Huldchinsky, have assumed that the rays of light which exercised the curative action in rickets were the ultra-violet, and such an assumption seemed almost necessary, since window glass filters out of sunlight the ultra-violet radiations, and children behind window glass are not protected from rickets. A multitude of observations and experiments have indicated that the rays of light which produce photochemical reactions lie at the ultra-violet end of the spectrum.

Hess, Pappenheimer and Weinstock (113) have shown that in the case of the rat the rays of light which protect against rickets lie in the ultra-violet zone and are about  $300\ \mu\mu$  in length, or shorter. The question exactly which are and which are not the active rays, is complex, since two factors must be considered—the length of the rays and their intensity. It is possible, for example, that rays of light which exert only a feeble effect in the cure of rickets may have great effect if supplied in great intensity as in sunlight. While the general conclusion of Hess, Pappenheimer and Weinstock is not to be doubted, large numbers of experiments must be made with both variables taken into consideration before it will be possible to consider this question as settled.

<sup>7</sup> Results of experiments by Dr. H. Laurens and Dr. G. F. Powers which have never been reported.

*When the organism is deprived of the influence of X in the food and of radiant energy defects in the diet become reflected in the blood.* This fact is one of the most important of those deduced from experiments on the rat. Diet 761 of McCollum, Simmonds, Shipley and the present writer contained 0.05 per cent calcium and 0.45 phosphorus. According to the analysis of Howland and Kramer (76), the serum of the animals on the diet contained 5.5 mgm. calcium and 8.0 phosphorus per 100 cc. blood. Cod liver oil administered for fourteen days raised the calcium to 8.2 mgm. Diet 618 contained 1.22 calcium and 0.3 phosphorus. The serum of rats on it showed 10.5 mgm. of calcium and 2.4 mgm. of phosphorus per 100 cc. blood. In five days cod liver oil raised the phosphorus to 5.7 mgm. per 100 cc. blood.

*In the absence of X and of radiant energy, rickets can be made to develop by altering the composition of the diet in several ways.* Undoubtedly the composition of the organic portion of the diet, quite apart from its content in X, exerts an important influence in determining the development or non-development of rickets. Unfortunately, the experiments of the New York group of investigators throw little light on this aspect of the question, because they used the same basal ration in all their experiments. McCollum, Simmonds, Shipley and the present writer, on the other hand, used a great variety of diets. Their experiments established the fact that the organic portion of the diet (quite apart from its content in X) *may* exert a determining influence. It seems probable that an excessive proportion of carbohydrate in the diet and an insufficient amount of protein or protein of poor quality favors the development of rickets, and, indeed, that any defects in the composition of the organic portion may favor its development if they are of such a nature as to make the diet fail to meet the nutritional needs, and at the same time do not prevent growth of the skeleton. Since no positive information exists to the contrary concerning the influence of the organic constituents of the diet (apart from X), one must at present assume that a faulty composition of the diet in respect to fats, carbohydrates and proteins acts chiefly in an indirect manner through its influence on the organism as a whole.

In contrast to the uncertainty which surrounds the question of the influence of the organic components of the diet, no doubt exists that the salt composition of the diet exercises a determining influence for or against the development of rickets. Rickets has been produced:

1. By diminishing the phosphorus content of the diet and administering calcium in the form of the carbonate or lactate or chloride in

disproportionately large quantities (62), (66), (68), (64). The rickets produced in this way is characterized by a low blood phosphorus and a histological condition corresponding to that ordinarily found in the human being.

2. By diminishing the calcium and maintaining the phosphorus content of the diet at a higher level than that of calcium (64), (77). The rickets resulting from diets of this kind has certain distinctive features. The arrangement in the metaphysis of the bone is more orderly than in the low phosphorus form of the disease; evidences of resorptive activity are marked; cells from the fixed tissues with basophilic granules lie scattered about in the immediate vicinity of the trabeculae. No doubt, however, can exist concerning the identity of this form of rickets with the rickets of the human being, and chemical analyses of the blood of the human being are bringing to light proofs of the existence of rickets in which the calcium is low.

3. By adding magnesium carbonate to the diet.<sup>8</sup> McCollum, Simmonds, Shipley and the present writer produced rickets of the greatest severity, by the addition to the diet of magnesium carbonate in quantities varying between 1 and 4 per cent. The rickets corresponded in its general features to the low phosphorus form of the disease. The magnesium carbonate was most effective in diets in which the phosphorus was low. If given in large amount, it had so deleterious an influence on the health of the animal that life was not continued for more than a few weeks.

4. By the addition of strontium carbonate to the diet (78). By the addition of strontium carbonate in amounts equalling 2 per cent, an extreme rickets of the low phosphorus variety was produced. Animals on this diet almost invariably developed paralysis of the hind legs. The condition seemed to be fundamentally different from that which Lehnerdt (79) obtained by feeding strontium. That investigator used diets relatively high in phosphorus. The effect of the strontium was complicated, therefore, by the effect of a high phosphorus.

Experience has shown that not all diets deficient in X and having unusual calcium phosphorus balances regularly produce typical rickets. Undoubtedly there are many factors in the diet about which nothing is known, and the question whether these factors are to be found in the organic portion of the diet or in the inorganic portion must remain unanswered pending further investigation. At any rate, in an im-

<sup>8</sup> These experiments have never been published.

portant paper just published, Zucker, Johnson and Barnett (75) report that by making the Sherman-Pappenheimer diet acid through the substitution of calcium chloride in equivalent quantities for the calcium lactate, the rickets-producing power of the diet was removed, and that by adding ammonium chloride in amounts equalling 2 per cent of the total ration its rickets-producing power was lost. A result, the reverse of the ones just recorded, was obtained when a diet which was made up of flour, egg albumin and a suitable salt mixture was used. This diet did not produce rickets. When, however, sodium carbonate was added in an amount equalling 2 per cent, this diet was converted into a rickets-producing one. The experiments of Zucker, Johnson and Barnett indicate that a determining factor for or against the development of rickets is the hydrogen ion concentration of the diet. The work just mentioned must be corroborated. McCollum, Simmonds, Shipley and the present writer succeeded in obtaining rickets of the low calcium variety when the ash of the diet was acid or alkaline. They obtained rickets of the low phosphorus variety when calcium chloride was used instead of calcium carbonate. The experiments of Zucker, Johnson and Barnett, however, are important, not alone from the fact that they indicate another way by which the equilibrium between the calcium and the phosphate ions of the blood may be upset so as to give rise to rickets, but also because they suggest that defects in the salt composition of the diet, other than the disproportion between the calcium and phosphorus, may be significant. When diets characterized by disproportions between the calcium and phosphorus are fed to animals in the absence of  $X$  and radiant energy, the resulting change in the levels of calcium and phosphorus of the blood is due to a direct action. Undoubtedly, however, the calcium ion or the phosphate ion of the blood can be depressed by indirect action.

*In the absence of  $X$  and radiant energy, rickets can be prevented by means of the diet.* The effect of the organic portion of the diet, quite apart from its content of  $X$ , on the development or non-development of rickets has already been discussed. Here it is necessary merely to restate the general principle that, if the organic portion of the diet is composed in such a way as best to meet the requirements of the animal and is supplied in sufficient amount, it undoubtedly contributes protection against rickets. Again it is necessary to emphasize the extreme limitations of our knowledge concerning the specific effects of the organic components of the diet.

The fact, however, that rickets can definitely be prevented by the salt composition of the diet is of great importance. For example, the salt mixture 185 devised by McCollum (66) effectually prevents the development of rickets when substituted for a salt mixture which leads to the development of rickets. Sherman and Pappenheimer, it will be remembered, converted their rickets-producing diet into one which did not produce rickets through the simple addition of 0.4 per cent alkaline potassium phosphate, which restored the calcium phosphorus ratio in the diet to one within normal limits. If the work of Zucker, Johnson and Barnett proves to be correct, it is possible to make the diet lose its property to produce rickets by making it acid. Doubtless, as our knowledge of the interrelationships between the diet and the production of rickets increases, it will be found that the disease can be prevented by means of the diet in a variety of ways.

Before leaving the subject, it is advisable to point out the influence of fat-soluble A on the development of rickets. In the diet used by Sherman and Pappenheimer, and in diet 3127 used by McCollum, Simmonds, Shipley and the present writer, the quantity of fat-soluble A present was insufficient to prevent the development of xerophthalmia, and rickets developed. It is possible, therefore, to obtain rickets, even though fat-soluble A in the diet is reduced to a minimum. The rickets never reaches the degree of development under that condition, however, which is possible when fat-soluble A is present in the diet. Apparently when a young, rapidly growing animal is suddenly placed on a diet which is defective in fat-soluble A and is incapable of maintaining growth, growth continues for a time as the result of what may be termed a growth momentum. During this period the salt defect in the diet, if such is present, manifests itself sooner than the defect in fat-soluble A. The new cartilage and bone tissue are formed without lime salts, and a state of rickets is brought about. Pappenheimer and his collaborators and McCollum, Simmonds, Shipley and the present writer found that small quantities of butter added to the diets deficient in fat-soluble A made the rickets more pronounced.

*Butter fat is exceedingly poor in its content of X as compared with cod liver oil.* Mellanby noted that butter fat was inferior to cod liver oil in its power to prevent rickets, and the only reason that Findlay, Patton and their associates did not fail entirely in the assault on Mellanby's position was the chance choice of butter fat rather than cod liver oil as the substance representing the "anti-rachitic factor." If they had used a potent cod liver oil in sufficient quantity, not one puppy would have become rachitic in any of their experiments.

The proof that butter fat is feeble in its power to cause lime salt deposition in rickets appeared early in the work of the American investigators. At the same meeting, May, 1921, Pappenheimer (80) announced that the addition of butter to the Sherman-Pappenheimer diet in the proportion of 0.2 gram per 100 grams of ration (an amount barely sufficient to prevent xerophthalmia) did not prevent the development of rickets, and the writer reported that butter fat, added to the rachitic diet in quantities equalling 10 per cent of the total ration, was insufficient for that purpose. McCollum (81), (73) also presented a paper which showed from a totally different standpoint the inferiority of butter fat as compared with cod liver oil. When the calcium of the diet was low, butter fat was markedly inferior to cod liver oil in its power to cause growth as well as to induce calcification of bone. Butter fat to 20 per cent of the diet failed to cause satisfactory growth in the presence of a marked calcium deficiency in the diet, whereas cod liver oil was effective in an amount equalling 3 per cent of the diet. In subsequent publications Pappenheimer, McCann and Zucker (65) proved that the addition of butter fat to the amount of 10 per cent of the diet neither prevents nor causes rickets. McCollum, Simmonds, Becker and Shipley (56) showed by means of the "line test," (82) that butter fat must be fed in proportions of 15 to 30 per cent of the diet in order to induce lime salt deposition in the cartilage.

*Cows' milk cannot be regarded as an "anti-rachitic" food.* The fact that butter fat is feeble in its anti-rachitic action is of practical importance; it means that cows' milk is not a strongly anti-rachitic food, and explains clinical experience which has taught on every side that a diet of cows' milk and rickets are entirely compatible. In this connection the work of Shipley, McCollum, Simmonds and the present writer (72), (73) are of significance. This work showed that cod liver oil possesses in far greater degree than butter fat the power to aid the animal in his struggle against the adverse conditions imposed by diets low in calcium. Evidently butter fat is designed to operate only in the medium in which it is contained.<sup>9</sup> It is possible that the particular combination of salts, protein, carbohydrate and fat which compose cows' milk may be such as actually to favor the development of rickets when introduced into the human organism. The fact cannot be forgotten that the great majority of children who develop rickets do so on diets of cows' milk.

*Will cod liver oil invariably cure rickets?* In the rickets produced in the rat by diets high or relatively high in calcium and low in phosphorus,

<sup>9</sup> View also expressed by Mellanby.

the low phosphorus form of rickets (62), (63), (64), (66), (68), (83) cod liver oil restores the histological condition in the bone essentially to the normal in from fourteen to twenty-one days. The trabeculae may be fewer than in the bones of the rat on a normal diet but are completely calcified. The provisional zone of cartilage is completely calcified. The bones of the skeleton remain smaller than in rats on satisfactory diets and in comparison with those of the latter are thin.

In the rickets produced by diets in which the calcium is low and the phosphorus higher, the low calcium form of rickets (64) (77), (83), cod liver oil never restores the finer structure of the bone to the normal. The trabeculae do not become completely calcified, and the provisional zone of cartilage is not always completely calcified. The arrangement of the cells in the proliferative zone of cartilage is, however, regular, showing that the orderly growth of the cartilage does not depend on calcium deposition between the columns of cells; the arrangement of the trabeculae in the subchondral portion of the bone is orderly. The trabeculae are greatly increased in number. The reason that cod liver oil does not restore the finer structure of the bone to the normal in the presence of a relative calcium defect, lies in the fact that the calcium depot of the body is in the skeleton, and it becomes necessary for the organism continually to draw upon it. Everywhere in the trabeculae the destruction of old bone and the formation of new, the *umbau*, go on with increased rapidity. Obviously a circulation of calcium is set up through the intermediary of the blood from parts of the bone in which it is not needed to the points where it is most needed, viz., the cartilage and adjacent portion of the shaft. The holes in the trabeculae, many of which contain calcified fragments and free osteoblasts, and the osteoid borders along the trabeculae are merely evidences of the conservation process which the organism resorts to in the face of a calcium deficiency. Cod liver oil cannot ever be thought of as initiating any new process in the bone, but merely as accelerating those which are natural to the organism (84), (85).

Though cod liver oil cannot be said, as judged by histological appearances, to restore the abnormal condition in the bone induced by calcium starvation to the normal one, it does so for all practical purposes, for the gross deformities of the skeleton disappear, and the bones become strong enough to meet the requirements of the animal. Only in rats of the second or third generation on low calcium diets, containing cod liver oil, do fractures regularly develop. In such animals 100

or more green-stick fractures have been counted in the ribs. The bones of the extremities remain exceedingly thin but intact.<sup>10</sup>

When both calcium and phosphorus are low in the diet, and cod liver oil is administered, the histological structure of the bone seems to be determined by the calcium deficiency and is most closely allied to that already described for the low calcium form of rickets.

When rickets is produced by the addition of strontium carbonate to the diet (78), a form of rickets which probably never occurs in the human being, cod liver oil exerts little influence. In the rickets induced by the administration of magnesium carbonate, also, cod liver oil exerts a limited influence. It is possible that the rickets from excessive quantities of magnesium in the food may occur in animals (86).

When Findlay (87) states that cod liver oil does not cure the rickets of the human being, one infers either that the cod liver oil used was not potent or was not actually taken. One can be certain that in the human being with rickets, with the possible exception of the premature infant, cod liver oil never fails to exert a curative action without change in the living conditions or diet.

*The effects and mode of operation of cod liver oil and radiant energy.* What cod liver oil and radiant energy actually accomplish is beautifully illustrated in experiments performed by Powers and Guy.<sup>11</sup> These investigators placed large series of rats, abundantly controlled and living in a medium of roomlight, on a basal ration which was defective in X, calcium and phosphorus. In the diets fed two sets of animals the calcium was made high and the phosphorus kept low. In the diets fed two other sets the calcium was kept low and the phosphorus was made high. When these diets had been administered long enough for the disproportions between the calcium and phosphorus to become reflected in the blood, as was determined by chemical examination, cod liver oil was administered. In those animals in which the calcium of the serum was low, cod liver oil caused the calcium to rise to the normal level, restoring a normal equilibrium. In those animals in which the inorganic phosphorus in the serum was low, cod liver oil caused the inorganic phosphorus to rise to a normal level, establishing a normal equilibrium. Obviously cod liver oil acted as a regulator of the mineral metabolism, at least in so far as the calcium and inorganic

<sup>10</sup> These experiments by McCollum, Simmonds, Shipley and the present writer have never been reported.

<sup>11</sup> These experiments have not yet been reported.

phosphorus are concerned, and one may infer that a function of cod liver oil and also of radiant energy is to maintain the normal salt equilibrium of the body in the presence of salt combinations of different complexities which are continually being absorbed from the food.

Though we may only conjecture concerning the mode of operation of cod liver oil and radiant energy in rickets, certain observations concerning their effects are suggestive. Animals on rickets-producing diets, which are given cod liver oil and kept in the sunlight, grow better, are more active, more nearly normal, and live longer than their control animals. Obviously the beneficial effect of the energy derived from the sun and of cod liver oil is not limited to the rachitic process. Also, the favorable effect of cod liver oil and radiant energy on rats receiving diets which give rise to rickets is maintained as long as either is supplied.<sup>12</sup> The examinations by Howland and Kramer (76) of the blood of the animals used in the experiments of McCollum, Shipley, Simmonds and the present writer have shown that cod liver oil maintains the level of the calcium in the blood near to the normal in rats kept for four generations on diets so deficient in calcium as to render the animals in a perpetual state of calcium starvation. Neither cod liver oil nor radiant energy supplies the required calcium or the phosphorus. It is an extraordinary fact, however, that either one can enable the rat, kept in a state of calcium or of phosphorus starvation, to obtain so good a utilization of the minimal quantities of calcium or of phosphorus supplied in the food as to maintain the level of the calcium or of the phosphorus in the body fluids almost at the normal. Without supplying either phosphorus or calcium radiant energy or X causes the organism to operate as if a requisite or almost requisite quantity of phosphorus or calcium were supplied. They do so by supplying something which makes the metabolism more efficient, i.e., they cause the organism to operate with increased economy. Neither the histological studies of the bones nor the chemical examinations of the blood indicate that either cod liver oil or light bring new processes into operation, but rather permit the organism to have full use of processes which were natural to it all the time, but were not effective.

<sup>12</sup> The question arose: Did not cod liver oil or radiant energy exert their favorable influence in rickets by making available to the organism depots of phosphate or calcium which previously had been inaccessible? On that supposition one would expect the rickets to recur when the depot of phosphate or calcium finally became exhausted, even though the administration of the cod liver oil was continued.

Radiant energy is a powerful oxidizing and reducing agent in the case of the simpler chemical compounds, and probably exerts an influence of some such general nature in man and animals. It communicates to the organism energy in some form which is apparently essential for normal metabolic activity, in particular in the growing organism. It is probable that cod liver oil operates in some such general way.

Apart from rickets cod liver oil and sunlight cannot be considered to have an equivalent action. Powers, Simmonds and the present writer (114) have shown that radiations from the mercury vapor quartz lamp have almost no influence in the prevention of xerophthalmia in the rat, whereas cod liver oil has a powerful preventive action. They found that sunlight and fresh air, on the other hand, delayed the development of xerophthalmia in some animals and in others prevented it over periods of four or five months. Obviously, however, sunlight and fresh air had kept the xerophthalmia only just submerged, since it quickly made its appearance when the animals were removed from sunlight to ordinary roomlight. Cod liver oil prevents xerophthalmia more easily than it prevents rickets; sunlight, however, prevents the development of xerophthalmia only with difficulty but readily prevents the development of rickets. The investigation just mentioned speaks rather in favor of the existence of at least two factors in cod liver oil, the one exercising a special influence in the prevention and cure of xerophthalmia and the underlying disturbance in metabolism, the other exercising a special influence in the maintenance of the normal salt equilibrium in the blood. It also shows that cod liver oil furnishes a factor the equivalent of which sunlight does not supply, or supplies in small amount. Abundant evidence exists that radiant energy produces effects which cod liver oil cannot bring about. So far as the influence on the mineral metabolism in rickets is concerned, however, their functions seem to overlap. It is difficult to conceive that *X* so abundantly contained in cod liver oil, and radiant energy which exercise this extraordinary influence on the metabolism of the organism, should be entirely unrelated. Doubtless the future will show that some intimate connection exists between them.

*Effects of starvation.* The attention of McCollum, Simmonds, Shipley and the present writer (115) was early called to the fact that animals which grew poorly and presumably ate poorly did not show rickets. The thought, therefore, suggested itself that starvation might excite healing. Accordingly, rats that had been rendered rachitic by means of diets low in phosphorus and fairly high in calcium carbonate

were starved for five day periods. When they were killed, beautiful deposits of lime salts comparable to those found in rats giving a positive "line test" to cod liver oil stretched across the border of the cartilage at the rachitic metaphysis. Observations which have the same significance were made by Pappenheimer and his collaborators.

The starving animal no longer carries the load of the unbalanced diet. His tissues, breaking down, liberate substances required to restore the normal equilibrium, including perhaps X itself. Many studies of the metabolism in starvation have shown that the body in that state uses its resources with the utmost economy. It is not strange, therefore, that under the influence of starvation the normal equilibrium reforms. The phenomenon has its analogue in the favorable influence of starvation on the metabolism in diabetes and other diseases.

*The relation of growth to the development of rickets.* For the development of manifest rickets and osteomalacia growth is essential. Both are diseases intimately concerned with the phenomenon of growth. The peculiar turn which the metabolism takes in rickets is probably dictated by the requirements of growth, and in this way is explained the frequency of rickets during the periods of rapid growth.

So many writers have pointed out the fact that malnourished infants do not become rachitic, that one need only mention the fact here. Sometimes, however, the subjects of extreme rickets are emaciated. Jundell (25) has been so impressed by the apparent relation between rickets and growth that he has revived the idea originally advanced by Glisson which ascribes rickets to over-eating (over-nutrition).

*Is the rickets produced experimentally in animals the same disease as rickets in the human being?* Findlay (60) questions whether the rickets induced in animals is the same disease as the rickets of the human being. In reply these facts may be cited: The lesions in the skeleton, both gross and minute, are identical; the pathological conditions found to exist in the blood are identical; the rickets produced experimentally in animals may or may not be accompanied by the symptoms of tetany, exactly as rickets in the human being may or may not be accompanied by tetany; the same remedial measures are effective in both. Not the slightest doubt can exist that the rickets produced experimentally in animals and the rickets occurring in human beings is the same disease.

THE CHEMICAL CHANGES OCCURRING IN RICKETS: *The changes in the blood occurring in rickets.* A great step forward was accomplished when Iversen and Lenstrup (116), and Howland and Kramer (117), quite independently, reported that in ordinary rickets (rickets uncomplicated

with tetany), the inorganic phosphorus in the blood is low. Howland and Kramer found that in non-rachitic infants the concentration of calcium is from 10 to 11 mgm. per 100 cc. of serum and of inorganic phosphorus about 5 mgm. During the period of active rickets the calcium concentration of the serum may be normal or slightly reduced, a result in agreement with that previously reported by Howland and Marriott (118). The inorganic phosphorus of the serum, however, is regularly reduced, in some cases to an extreme degree, for example to 1.0 mgm. With the exception of that form of tetany which is induced in infants through the administration of the sodium carbonates or by over-ventilation of the lungs, infantile tetany must be regarded as a symptom-complex occurring in rickets. It is, therefore, necessary to inquire into the conditions prevailing in the blood in tetany. Howland and Marriott (118) some time ago found that the calcium of the blood is considerably reduced in infantile tetany, exactly as in the tetany of animals caused by extirpation of the parathyroid glands. During active symptoms the calcium of the serum may fall to 3.5 mgm. per 100 cc. of blood. The inorganic phosphorus is, however, usually "normal" varying from 3.6 to 5.8 mgm. per 100 cc. The concentration of sodium and magnesium was found to be essentially "normal," that of potassium slightly increased. When calcium is administered in tetany in the form of calcium chloride, the calcium in the blood serum rises. In the human being and also in the rat the rise of the calcium, however, is frequently associated with a fall in the inorganic phosphorus. These findings in the blood in ordinary rickets and in rickets accompanied by tetany have received abundant confirmation (Findlay, Paton and Sharpe (1), Hess (119), György (120), von Meysenbug (121) and others).

Iversen and Lenstrup reported that the inorganic phosphorus of the blood was increased after the administration of cod liver oil. Howland and Kramer also reported its regular increase after the administration of cod liver oil to the normal level and "often somewhat above this." Apparently the rise in the inorganic phosphorus of the blood began to be apparent after fourteen days of treatment and did not become complete until four to six weeks had elapsed. Radiant energy exerts an influence on the level of the inorganic phosphorus of the blood in rickets which is identical with that of cod liver oil. Hess and Gutman, (107) Hess, Unger and Pappenheimer (110) and Howland and Kramer (76) have reported that radiant energy, whether derived from the sun or artificial sources rich in ultra-violet radiations, cause the low inorganic phosphorus of the serum in rickets to rise.

In this connection the reports of the chemical examinations of the blood made by Howland and Kramer (76) on the rats used in the experiments of McCollum, Simmonds, Shipley, and the present writer are of the greatest interest. The findings in the blood corresponded in a wonderful manner with the histological findings and have both supplied new information and given splendid support to the conclusions drawn from the latter. The chemical examinations of the blood showed: *a*, that the concentration of the inorganic phosphorus and calcium of the serum cannot be made to exceed the concentrations regarded as normal; *b*, that when the concentration of either calcium or inorganic phosphorus in the serum is low, it may be increased by increasing the amount of the respective element in the diet; *c*, that when the inorganic phosphate of the serum is low it may be increased by starvation, the addition of phosphorus to the diet in organic or inorganic form or of various fats (the fish oils and butter fat), and by exposure to radiations of the requisite quality. The examinations of the blood also indicated that cod liver oil raises the calcium of the serum in the rat when the latter is low.

*The metabolism in rickets.* Knowledge concerning the metabolism of rickets is so confused that the wisdom of discussing it beyond the point of obtaining a general orientation seems doubtful. Reviews of the subject will be found by Orgler (122), Lehnert (123), and Paton, Sharpe and Findlay (1). Two splendid studies to which the reader is especially referred are those by Telfer (124) and Hamilton (125). The one by Hamilton was made on the premature rachitic infant.

The disturbances in the salt equilibrium in the blood which characterize rickets have already been described. The total ash of the bones is greatly reduced. For example, Telfer found the mineral matter of the dried bones of the leg of a rachitic dog to be 17.7 per cent, as compared with 44.9 per cent in the normal animal. Brubacher (126) gives similar figures for the rachitic and normal child. Another important fact is that in the rachitic subject the ratio of calcium to phosphorus in the bone does not differ materially from that in the bone of normal individuals. Both Telfer and Hamilton have shown that in rickets the amounts of calcium and phosphorus retained are less than in healthy persons. This point in the metabolism of rickets has been definitely established. Schabad (127) has reported negative calcium and phosphorus balances in rickets and it is probable that in rickets negative balances may occur. Another interesting point brought out by Telfer is that the proportion of phosphorus retained is less than that of cal-

cium. A fact which has especial importance in regard to the metabolism of the premature infant with rickets and all infants on diets of breast milk was brought out by Hamilton. He showed that the normal infant excretes about 0.200 gram of calcium oxide daily, regardless of the intake or weight. For an adequate calcium retention to occur, therefore, it is necessary that considerably more than that amount be supplied in the food. There is no good evidence that fat, protein, or carbohydrate, considered as such, influence calcium metabolism. Schabad (128), Schloss (129) and Orgler (122) have reported that cod liver oil increases calcium retention; Freund (130), Orgler and others have performed experiments tending to show that milk fat decreases calcium retention. Telfer failed to find that either butter fat or cod liver oil influenced calcium retention.

The metabolism of rickets must be studied anew. In most investigations the periods of study have been too short, and the condition of the child during the experimental period has not been sufficiently taken into account. The fact that X in cod liver oil and other substances does not seem to produce its effect until a period of days has elapsed has not been taken into account. In view of the remarkable influence of radiant energy on the mineral metabolism, fresh experiments on normal infants ought to be carried out in order to determine normal standards.

There are several explanations for the diminution in the retention of calcium and phosphorus in rickets. One is that the calcium and the phosphorus are not absorbed from the intestinal tract. On this supposition it is likely that either the digestive functions are disturbed in some peculiar way, or else the combination of food substances is such that insoluble products incapable of absorption are formed, for example, tri-calcium phosphate or calcium soaps. Another possibility is that the calcium and phosphorus are absorbed, but, on account of abnormal conditions in the internal metabolism, are not allowed to accumulate in the blood; they are either driven out of the body or are used to carry out some other substance. Under this latter supposition it is necessary to suppose the primary disturbance to be in the internal metabolism. In either case it seems altogether probable that the deposition of lime salts in the bones is determined chiefly, if not entirely, by the products of the concentration of the calcium and phosphate ions in the circulating fluid as Howland and Kramer (131) have indicated. When the concentrations of calcium and phosphate in the blood increase sufficiently, the deposition of lime salts apparently

occurs. Telfer and Zucker seem to incline to the view first mentioned. If, however, the calcium and phosphorus are not absorbed from the digestive tract, is it not because the internal metabolism does not demand their absorption? Even if they are not absorbed, it does not follow that the disturbance is not in the internal metabolism which may have failed to create the necessary demand. It seems easier to explain the action of radiant energy and also of cod liver oil in rickets on the ground that the metabolism of the whole body is altered, rather than on the basis of a peculiar composition of the food or a local disturbance in the digestive system. We should beware, however, of drawing positive conclusions in a subject about which so little is known. Moreover we ought to remember that such a disturbance in metabolism as the one occurring in rickets probably does not always come about in the same way.

*The social and living conditions of rachitic children.* Rickets occurs most commonly among the children of the poor, who eat poor food and spend much of their time indoors in poorly-lighted, badly-ventilated, dark rooms. These are facts emphasized by Kassowitz and many others. It is important to remember, however, that rickets not infrequently makes its appearance in families of means living under conditions of hygiene and diet which have been regarded as above criticism. Instead of generalizing further, it seems advisable to give brief résumés of the two best studies on this subject, the one by Ferguson (24) in Glasgow, the other by Hutchinson and Shah (132) in India.

Ferguson investigated two hundred families in which marked rickets existed, one hundred and fifty families in which there was mild rickets, and one hundred families in which the rickets appeared to have been cured. She compared the conditions found with those present in two hundred families in which the children were free from rickets. The chief points brought out were: Inadequate air and exercise appeared to be potent factors in determining the onset of rickets. In the rachitic families the social conditions were somewhat the worse. The average number of persons per room was almost one greater; the cubic space per person was 32 per cent less; the houses were less cleanly, and the children were taken out of doors in the fresh air considerably less. ("Over 40 per cent of the rachitic children had not been taken out, while only 4 per cent of the non-rachitic children had been confined indoors.") Ferguson's study of the dietaries in the rachitic and non-rachitic families was not altogether satisfactory. In general, the diets in the non-rachitic families had about the same energy value as

those in the rachitic families. In the non-rachitic families the average amount of fat in the diet was 10.7 per cent higher; the consumption of milk was somewhat greater; that of fish and eggs double; and of margarin or butter slightly greater than in the rachitic families. Though Ferguson minimized the differences in the diets in the two groups, the diet of the non-rachitic families was the better. The study by Ferguson was an extension and corroboration of a similar study made by Findlay several years previous.

In their most remarkable investigation of the effect of hygiene versus diet upon the development of rickets, Hutchinson and Shah made direct comparisons of diets and living conditions among the Mahomedans and Hindus of the laboring classes with those prevailing among the higher caste Mahomedans and Hindus, among whom the practice of the "purdah" was usual. The women, as well as the men of the laboring classes, worked in the fields, and while at work left their young infants at some nearby point in the open air. The houses of this class were "one roomed, ill built huts with a hole in the roof for a chimney." Among the higher caste Hindus and Mahomedans the infant remained, as a rule, for the first six months of his life with his mother in a semi-dark, ill ventilated room in the interior of the house, and ordinarily remained indoors until able to walk and look after himself. The women usually married at the age of twelve, entered "purdah" and from that time on left the house only for short periods on special occasions. The houses were built in such a way as to admit little light and air. The dietaries of the higher caste Hindus and Mahomedans were considerably better than the dietaries of the laboring class. They contained more animal fat, butter, eggs and milk. Fresh vegetables figured little in the dietaries of either. The point of the investigation was that rickets occurred scarcely at all among the infants of the laborers, whereas it was exceedingly common among the children of the upper caste families. Moreover, among the women of the upper castes late rickets was frequently encountered.

In reality the investigations of Hutchinson and Shah showed merely that sunlight prevented the development of rickets. The diets of both castes were deficient in the factor X and poorly constituted. The children and women of the laboring class were protected by sunlight. Those of the higher castes lacking the influence of sunlight developed the disease.

*Diets of rachitic children.* Until quite recently it was taught that diets in which the carbohydrate was in excess, the fat deficient, and

perhaps also the protein, produced rickets. This view arose from the observations of physicians that rickets was particularly apt to appear in children receiving condensed milk and proprietary foods. At the same time the theory found support in the reports of experiments by Bland-Sutton at the Zoölogical Garden. The so-called experiments of Bland-Sutton have had an influence which apparently they have not merited. Cheadle and Poynton (133) wrote concerning them in 1908: "The history of these lion cubs is very significant; with the exception of a single litter, suckled by the dam ten years before, the cubs brought up on horse-flesh in this way invariably died,—the cause of death being, as invariably, extreme rickets. More than twenty litters had been lost in this way. The feeding of the last litter of lion cubs was begun in the usual fashion. The dam had very little milk, and at the end of two weeks the cubs were weaned entirely and were then put on horse-flesh as usual. They quickly became rickety, and the muscular weakness, as well as bony deformity, were extreme. The malady advanced rapidly and one cub died. Then, by the advice of Mr. Bland-Sutton, milk, pounded bones, and cod liver oil were added to the raw meat, which was continued exactly as before; they were kept in the same dens with the same amount of warmth and light and air, and, with the single exception of the addition to the diet, no change of any kind was made in the regimen. The change in nutrition which followed was immediate and remarkable; in three months all signs of rickets had disappeared, and the animals grew up strong and healthy—a unique event in the history of the society. The experiment seems a crucial one, and decisive as to the part played by fat and bone salts, with some casein and lactose, in the production and cure of rickets" (pp. 85 and 86).

Findlay (87) wrote concerning these experiments in 1921: "Bland-Sutton has kindly supplied me with details of his experiences with rickets in young carnivora in the London Zoölogical Gardens, experiences which have been extensively quoted in the medical literature, but which were never published in detail by him. His attention has been drawn to the difficulty of rearing young carnivora in confinement. They 'were often born with cleft palate, and those that survived soon developed rickets.'" To remedy these things, he advised that the adult lions should be given goat flesh and bones twice weekly instead of being fed entirely on horse-flesh (the bones of horses being very hard), and that the young be given cod liver oil to lick. As a result of these changes in diet, some of the lion whelps were born without cleft palate,

and others, which grew up to adult life, presented remarkable rickety changes in their skulls. Such experiments do not justify the conclusions regarding the influence of fat in rickets that have been based on them by his disciples, and I know that within recent years Bland-Sutton has held the opinion that a gastro-intestinal disturbance, probably of a microbial nature, is the initial cause of the disease' (pp. 152 and 153).

Few systematic investigations of the diets of rachitic children have been made. As just mentioned, the investigations of Ferguson indicated that the diets in "non-rachitic" families were superior to those in the "rachitic" families, and those of Hutchinson and Shah showed that the diets in the "rachitic" families were better than in the "non-rachitic."

In January, 1920, Hess and Unger (134) put to the test upon the infant the diet which Mellanby had used with so much success for the production of rickets in puppies. They gave to five infants between four and nine months of age a diet composed of 180 grams krystalak, the fat content of which was less than 0.2 per cent, 30 grams cane sugar, 10 cc. orange juice, 30 cc. autolyzed yeast and 30 cc. cottonseed oil. The infants did not develop symptoms of rickets during observation periods of five to ten months. The experiments were undertaken in order to determine the influence of a deficiency in the diet of fat-soluble A on the development of rickets in the human being. From that standpoint they were not of great value. Fat-soluble A was still present in the food, and clinical observation alone did not make the freedom from rickets certain. It is interesting, however, to learn that this diet seemed to have a greater protective value than diets containing considerable amounts of butter fat, perhaps because of the very deficiency in fat-soluble A.

Chick, Dalyell, Hume, Mackay and Smith (105) have reported concerning the effects of two diets used as standards in Pirquet's clinic, Vienna. The first diet contained about two-thirds to one-half of its total energy in the form of sugar. It was, therefore, high in carbohydrate (65 per cent), relatively low in milk and, hence, milk fat (24 per cent), and in fat-soluble A, especially as the milk used was from stall-fed cows and found to be low in vitamine A by actual test; it was also low in protein (11 per cent). The second diet consisted of full cream dried milk diluted so as to yield 13 per cent solids. It contained protein (20 per cent), fat (45 per cent) and carbohydrate (35 per cent). Additions of cereals were made for older children, and cod liver oil was given to the younger infants. The addition of the

cod liver oil to the second diet was most unfortunate because it destroyed completely the value of any comparative data. It was found, as was to be expected, that the second diet (plus cod liver oil) protected against the development of rickets. The first diet proved curative when cod liver oil was added to it. The most important development from the study was the discovery that children on the first diet (when cod liver oil was not added) developed rickets during the winter and spring in spite of excellent hospital conditions, but did not develop it during the summer.

Several years ago McClure, and subsequently Powers,<sup>13</sup> analyzed the dietaries of infants and young children with rickets who entered Johns Hopkins Hospital. They found that infants and young children developed rickets on all foods ordinarily given. Negro children, who had not shown marked evidences of the disease while being breast fed, developed it in a severe form between the ages of ten months and two years, when they ate table food. The diets were composed of bread, syrup, candy, cake, potato, and little milk. It was fairly common to find infants on diets of sweetened condensed milk who had developed rickets, and others who did not show a trace of the disease. Several children with extreme rachitic involvement of the thorax and multiple fractures had received only diluted cows' milk, and in Dunham, Willis and Guy's<sup>14</sup> series of cases of very early rickets, eight of ten artificially fed babies were receiving such diets. In one extraordinarily severe case of rickets in a negro child the diet consisted mainly of toast and tea. It was common to see rickets in children whose diets of cows' milk, sugar, or starch mixtures had been prolonged into the second year. Hess (135), (136) thinks children are particularly liable to develop rickets on diets of protein milk.

One may state positively that rickets develops on all milk mixtures and on almost all foods or combinations of foods ordinarily given to children. In general, however, the diets of rachitic children, particularly of older children, are open to criticism. Either there is an excessive amount of carbohydrate or an insufficient amount of protein or of fat, or there is insufficient carbohydrate, as in simple dilutions of whole milk, or the protein is of poor quality, or the food value of the diet is below the requirements, or the diet is poor in calcium or in its total salts, or is unsuitable for the digestion or for the age, as when mixtures of milk and carbohydrate are continued into the second year.

<sup>13</sup> These investigations were never reported.

<sup>14</sup> These investigations will soon be reported.

Analysis of the diets of rachitic children, however, does not indicate that salt defects, such as seem to be required for the production of rickets in the rat, are present. In occasional instances the total salts may be low (the protein in that case is deficient also), or the calcium may be low, as in the diets of the negro children previously mentioned. The majority of the diets, however, on which children develop rickets, are based on cows' milk, and the salt composition is that of cows' milk. On the other hand, children may receive diets open to the greatest criticism from several standpoints and yet not develop rickets. Children on diets of carbohydrate alone do not develop rickets. The poorest diets will not cause rickets if they are supplemented with cod liver oil, or if the child is protected by sunlight.

One fact of great importance may be mentioned here. The older the subject of rickets, the greater the probability of marked defects in the diet. In old rachitic children the diet is almost invariably found to be defective.

Rickets occurs in breast-fed children with much greater frequency than generally supposed. A large percentage of negro babies who are breast-fed show signs of rickets, and many breast-fed white children of cities are affected. Rickets, however, does not usually develop in white babies, if breast-fed, and rarely manifests itself in breast-fed babies in severe forms. Findlay and Ferguson found rickets as common in children who had been breast-fed as in those who had never been breast-fed, an observation showing at least that breast feeding confers no lasting protection. Rickets in the breast-fed child is often accompanied by tetany.

A study was made by Hess and Unger (137) of the diets of the negro mother in a negro district of New York City where rickets was exceedingly prevalent among breast-fed children. The negroes were for the most part immigrants from the West Indies where they had lived an out-of-door life and consumed a diet consisting mainly of vegetables, eaten fresh, and fruits. In New York City they lived chiefly on meat or fish, rice and potato, tea, coffee, and cereal. Milk was taken in small quantity and fruit rarely. The vegetables which were taken in small amount were usually canned. In New York City the women lived indoor lives. The striking points brought out in the study were that the mothers had exchanged an out-of-door life in the tropics for an indoor life in the temperate zone, and a dietary in which green vegetables and green leaves figured largely for one in which those articles of food were almost entirely lacking.

In by far the most accurate study thus far made of the dietaries of the nursing mothers of rachitic children, Dunham, Willis and Guy<sup>15</sup> have found that the diets both during pregnancy and lactation are almost always poor and open to a variety of criticism. In the majority of instances the caloric value of the diet was under the requirement for mother plus child, and fresh vegetables and fruit were almost entirely lacking. In five of eleven instances the diet was low in calcium and in two instances in phosphorus. In the one instance in which the amount of green vegetables in the dietary was abundant, the total value was only 1125 calories, and the total phosphorus was under the minimum. It should be added that almost all the mothers of the rachitic infants led indoor lives.

In this connection the observation made by Siegert (30) that the breast-fed infants of rachitic mothers usually have rickets may be significant. Though the conditions of hygiene and diet which caused rickets in the infant do not produce the disease in the mother, they may be conceived of as affecting her to the extent of rendering her milk deficient in its protective qualities. Certainly the studies of Hess and Unger and of Dunham, Willis and Guy show that women receiving diets deficient in fresh vegetables and other foods containing fat-soluble A and probably X and living away from sunlight, do not yield breast milk which protects their offspring from the development of rickets. De Wesselow (138) and v. Meysenbug (121) have shown that the milk of the mothers of rachitic infants is not lacking in either calcium or phosphorus.

DISCUSSION. As the result of clinical observation and investigation it has become clear that two factors exist, the one in radiant energy, the other in an unknown form in certain foods, either of which is capable of preventing rickets from developing or from continuing, if already established. It has become equally clear, also, that only when the organism is deprived of the influences of radiant energy and of X, can rickets develop. Recent advances in knowledge have failed entirely to disclose the nature of vitamins, or their mode of operation in the organism or the mode of operation of the ultra-violet radiations of the environment. They have revealed, however, certain of the effects upon the organism of radiant energy and of X; both exercise a stimulatory or a regulatory influence on the metabolism of the body, in particular of the calcium and phosphorus. One at least of their functions appears to be the protection of the organism from the dangers

<sup>15</sup> This study will soon be reported.

attendant on the absorption and entrance into the blood of substances which might disturb its salt balance. Moreover, increased knowledge has indicated that the rôle played by radiant energy and  $X$  in the maintenance of the normal salt metabolism is of the utmost importance, and that the organism is dependent on the energy of the sun's rays or of their equivalent in the food to an extent little appreciated. The facts enumerated indicate beyond a doubt that rickets is a deficiency disease. Moreover, they settle for all time the differences of opinion between the English and the Scottish schools concerning the cause of rickets. Rickets is a dietetic disease, since, for its development the diet must be insufficiently supplied with the factor  $X$ . At the same time it is a disease of defective hygiene, since for its development there must be an insufficient supply in the environment of the radiant energy which exerts the protective action. The facts just mentioned also explain the disagreement among the students of rickets, the world over, concerning the causation of the disease. Obviously two factors have been concerned, but all investigators have proceeded on the assumption that only one factor was concerned, and have perceived so clearly the existence of the one that they have denied the possibility of the existence of the other.

As stated, radiant energy and  $X$  in the food exert only a protective or curative and regulatory influence. The question naturally arises, therefore, are there factors in the diet or elsewhere which actually can *bring about* the development of rickets? Experiments have proved that in the rat the food, quite apart from  $X$ , can precipitate the development of rickets (in the absence of protective radiations), or stop its development. In the case of the rat, therefore, no doubt exists that the food can exert a determining influence either for the development of rickets or against its development. Can the diet, then, exert a similar determining influence in the human being? Obviously absolute knowledge is lacking. Not the slightest doubt, however, can be entertained that diets such as do actually produce rickets in the rat would produce rickets in the human being under the same conditions; also, that diets such as prevent the development of rickets in the rat would prevent the development of rickets in the human being. The evidence at our disposal indicates that the human being is more susceptible to the development of rickets than the rat.

Since, then, it seems certain that the diet (apart from its content of  $X$ ) can exert a determining influence for or against the development of rickets in the human being, it is necessary next to inquire whether

the diet in the human being *does* exert this determining influence. Do the studies of the diets of children with rickets reveal defects which appear to be capable of precipitating the disease? The studies already referred to indicate that such abnormalities in the salt composition of the diet as seems necessary for the production of rickets in the rat are not ordinarily present in the diets of rachitic children. In some cases of rickets, in particular when older children or adults are affected, defects in the salt composition of the diet akin to those necessary for the production of rickets in the rat may be found. In a great majority of instances, however, variations in the calcium and phosphorus and in the reaction of the diets fall within limits which according to present standards must be regarded as normal. The point which stands out with great clearness in the study of the diets of children suffering from rickets is that no single fault (apart from a possible insufficiency in *X*) can be found which is common to all. The defects appear to be of various kinds and of such nature as rather to predispose to the development of rickets than actually to cause its development. It is true also that the diets on which some children develop rickets seem to be well constituted and do not cause rickets in others.

In this connection a word of warning is required. Knowledge concerning the relationship of different food substances to the organism is in its infancy. Defects in the diet of such nature as actually to be productive of rickets may be present in the diets of all rachitic children. In our present ignorance, however, one cannot recognize them.

Since the analysis of the diets of children developing rickets fails to reveal a constant defect or defects which appear to be capable of *bringing on* the disease, must it not be thought that the human organism is so dependent on the regulatory influence of radiant energy or of *X*, that rickets can develop even when the diet is well constituted,—in other words, that the human organism is extraordinarily susceptible to rickets? No single defect (aside from a possible insufficiency of *X*) is common to the diets of all rachitic children. Some infants develop rickets on foods which do not produce rickets in others. Infants may develop rickets even when a diet is composed of breast milk. Premature infants usually develop rickets, even when the food is breast milk. These facts make it necessary to conclude that the human organism is peculiarly dependent on the presence of radiant energy or its equivalent in the food, and that rickets may develop when the organism is deprived of them, even though the diet be what is commonly considered well constituted.

If the view just stated is true, then rickets is purely a deficiency disease. Is such a conception untenable? Of course, one would not expect to find the relationship between rickets and the diet so obvious as between scurvy and the diet, since in the case of rickets the deficiency in  $X$  can be compensated for by radiant energy in the environment. Yet if rickets and scurvy are compared, numerous points of similarity are found. In both diseases the incidence among infants is much greater than among adults. Under unusual circumstances great groups of adults have become the victims of scurvy, and, during the great war, numbers of adults in Austria and Germany became victims of osteomalacia. One reason that rickets occurs less commonly in adults than in children is that the adult has a more varied diet than has the child. The chief reason, however, lies in the fact that the adult resists far more those influences producing rickets than does the child. All who have attempted the experimental production of rickets in animals are agreed that old animals are more resistant than young animals to the development of rickets, and that rickets tends to undergo spontaneous cure with increasing age. As already pointed out, the younger the organism, the greater the dependence on radiant energy in the environment or on the factor  $X$  in the food, and the more intimate the relationship between these factors and the phenomenon of growth. Growth, however, never entirely ceases. The formation of new tissue and the destruction of old goes on continuously, even in extreme old age. Rickets differs from the other deficiency diseases, so far as present knowledge extends, in that the deficiency must be two fold.

There are two great arguments in favor of the conception that rickets is a deficiency disease. One argument lies in the following fact: The striking features in the occurrence and associations of rickets can be explained by the occurrence of radiant energy in the environment or of  $X$  in the food. The negro and Italian children in American cities are especially susceptible to rickets because the pigment of their skin partially insulates them, so to speak, from the sun's rays, and makes them derive less benefit from that source than do fair skinned children. The diets of the negro and Italian child in our American cities are notoriously lacking in those foods which carry fat-soluble A and presumably  $X$ . The negro child in Africa does not develop rickets because he lives out of doors and is nursed by a mother who eats food presumably rich in  $X$  (green leaves). Chinese and Japanese children do not develop rickets because they live out of doors and are breast-fed by mothers whose diets contain foods rich in fat-soluble A and presumably

in *X*. The children of the Esquimaux do not develop rickets, though they live in dark huts and for one-half of the year are in the Arctic twilight, because they are breast-fed by mothers who eat large quantities of fats abounding in *X* and are weaned to diets containing *X*. The seasonal variation of rickets is to be explained on the ground of the seasonal variation in the richness of the solar spectrum in ultraviolet radiations, as pointed out by Hess, but, also, by the seasonal variations in foods which contain *X*. The incidence of rickets varies with the deficiency in radiant energy and *X*, and not with any other factors which our present knowledge permits us to pick out. The association of rickets with scurvy is also in accord with the theory that rickets is a deficiency disease. It will probably be shown that a whole group of foods which are rich in the antiscorbutic vitamine are also rich in *X*.

The other argument which amounts to proof is as follows:—The addition of *X* to the diet or of radiant energy to the environment invariably cures rickets, if established, or prevents its development without change of the diet or conditions of life.

The whole subject of the etiology of rickets is extraordinarily complex, and the clear presentation of the facts, not to mention the correct interpretation, is most difficult. At the risk of falling into pitfalls, however, it seems wise to attempt to draw a parallelism between rickets and diabetes,<sup>16</sup> which will make clear the views of the present writer. Diabetes is a deficiency disease in the sense that an internal secretion essential for the maintenance of normal metabolism is deficient. Rickets is a deficiency disease in the sense that radiant energy and a factor *X* are deficient. Diabetes is a disturbance in the carbohydrate metabolism. Rickets, as we know it, is a disturbance in the metabolism involving in particular the calcium and phosphorus. It is possible to influence diabetes by means of the diet in two ways: directly, by feeding diets rich in carbohydrate, substances which the organism finds it most difficult to metabolize; and indirectly, by diets which are unsuitable in a general sense in that they do not meet the general requirements of the organism. In like manner, it is possible to influence rickets by means of the diet in two ways: directly, by feeding diets having specific salt defects or defects of an unknown nature which load down the disabled mechanism governing the salt regulation of the body; and indirectly, by diets which are unsuitable for the general requirements of the organism. In both diseases the diet can increase or diminish the metabolic disturbance according as it

<sup>16</sup> The differences between rickets and diabetes are of course appreciated.

strains or spares the metabolism at its weak point; in both diseases the diet can increase or diminish the metabolic disturbance according as it fails or succeeds in meeting the general requirements of metabolism. In diabetes the diet is not the cause of the disease; the cause lies in an insufficiency in the internal secretion of the pancreas. So, also, in rickets the diet (considered apart from *X*) is not the cause of the disease. The cause lies in a deficiency in the regulatory influence of radiant energy or the unknown factor in the food. In both diseases, the diet exercises a profound influence, not in bringing the disease into being, but in determining the extent and character of its manifestations. It is well known that a whole variety of conditions exerts an unfavorable influence in diabetes. Among them may be mentioned infection, fatigue, lack of muscular exercise, bad hygienic surroundings, exposure to cold, mental anxiety, etc. These conditions exert their unfavorable influence in diabetes because they weigh down an organism already handicapped by a disabled mechanism. In exactly the same way confinement, infection, bad hygienic surroundings (considered apart from the absence of radiant energy), exercise a deleterious influence in rickets, further weakening an organism already suffering from a disabled metabolism. Since rickets is a disease of metabolism and the substances to be metabolized are the foods, the diet can exert a more direct influence than can conditions such as those mentioned, with the exception of infection.

The general character of the relationship of infantile tetany to rickets has become apparent, but the exact nature of the metabolic disturbance necessary for the development of tetany continues to remain obscure. Apparently the disturbance in the salt equilibrium of the blood in rickets occurs chiefly in two forms: in one the calcium is low and the inorganic phosphorus somewhere near the normal; in the other the phosphorus is low and the calcium is somewhere near the normal. Between the two are innumerable gradations. Tetany is a symptom-complex which occurs in rickets when the salt equilibrium in the blood happens to be of a kind which sets the nervous system in a state of hyper-excitability. Apparently the disturbance in the salt equilibrium in rickets which is provocative of the symptom-complex, infantile tetany, is the one characterized by low calcium and it is with the low calcium form of rickets that manifest tetany is associated. If tetany can be considered a disease, then it is the same deficiency disease as rickets; it is cured and prevented by exactly the same means and differs from rickets only in the fact that the salt equilibrium in the blood happens to assume a special form or forms.

No treatment of the etiology of rickets can be complete without a discussion of the peculiar susceptibility of the premature infant to rickets and tetany. Premature infants are usually kept in poorly ventilated rooms which are often super-heated and dark; they are closely wrapped and are placed less frequently than are children born at term, in fresh air and sunlight. These facts alone are not sufficient to explain the frequency of the occurrence of rickets. The premature infant differs from the baby born at term in several particulars. He has low basal metabolism and is able neither to generate the heat necessary for nor to maintain normal body temperature; he has little subcutaneous fat and metabolizes fat poorly; in proportion to his size he grows with great rapidity. Hamilton has made important observations on the metabolism of premature infants. During the last three months of fetal life a storage of calcium takes place in the body of the fetus and during the first few months of life after birth the infant is obliged to draw on this calcium depot. In contrast to the child born at term, the premature infant has no calcium depot (or a deficient one) and is destined to suffer from calcium-want provided calcium is not abundantly supplied in the food. The calcium which the premature infant receives in his breast milk, however, is not greatly in excess of the amount actually excreted. Hamilton's investigations suggest, therefore, that the frequent occurrence of rickets in the premature baby may be caused by a deficiency in calcium and possibly in phosphorus.

The suggestion of Hamilton may furnish part of the explanation. It seems probable, however, that the great susceptibility of the premature infant to rickets results chiefly from the immaturity of the organism and great impetus for growth. The premature infant cannot be regarded as normal and indeed does not overtake the child of the same conceptional age until five or six years have elapsed. Moreover, his growth stimulus is enormous. The premature infant must, therefore, be far more dependent on radiant energy and on *X* than the child born at term and suffers more rapidly and severely when deprived of them. The child of four is more susceptible to rickets and develops the disease more rapidly than the adult, the child of one year than the child of four, the new-born baby than the infant of one year, the premature baby than the infant born at term, and the very small premature baby than the larger premature baby. According to Yllpö very small premature infants always develop rickets. The susceptibility to rickets varies inversely, therefore, with the age and culminates in the premature infant. To state the fact in another way, the susceptibility to rickets varies directly with

the youngness of the organism or the speed of new tissue formation. A theory similar to the one just presented has been advanced by Yllpö to explain the frequency of occurrence of anemia in the new-born infant. Doubtless rickets and anemia are sister conditions and both are expression of the inability of the organism properly to discharge its metabolic functions when thrown on its own resources in an unfriendly environment without being properly prepared.

In 1906 Hansemann wrote one of the great papers on rickets, by far the greatest written on the etiology of the disease. Hansemann perceived that man in his struggle for existence against the forces of nature had created a whole fabric of abnormal conditions, and in so doing had altered his own habits and ways of living as completely as he had altered the habits and ways of living of the domestic animals which he had made dependent upon him. "Domestication," wrote Hansemann, "does not relate merely to those things which pertain to the house, as the derivation of the word might indicate, but refers to every effort on the part of man to further the survival of the race and of the individual by artificial means and to aid in the struggle against the forces of nature. By this definition it becomes at once apparent that not alone does man domesticate animals but has domesticated himself, a fact clearly recognized by Darwin." According to Hansemann, rickets is the direct result of domestication and a penalty that man pays for the artificial structure which he has created. The writers on rickets seem to have assumed that Hansemann's conception referred only to the environmental conditions of the individual; clearly his conception included also the food. The food which domesticated man eats is as far removed from the food furnished by nature than the conditions under which he lives from the natural conditions. Recent progress in the understanding of rickets has served merely to give Hansemann's conception more exact definition. Rickets is indeed a price paid by man for his abandonment of a life out-of-doors and a natural diet for a life in houses and a diet of denatured foodstuffs; it is a sign of the operation of the immutable law of nature that nothing out of accord with her shall flourish.

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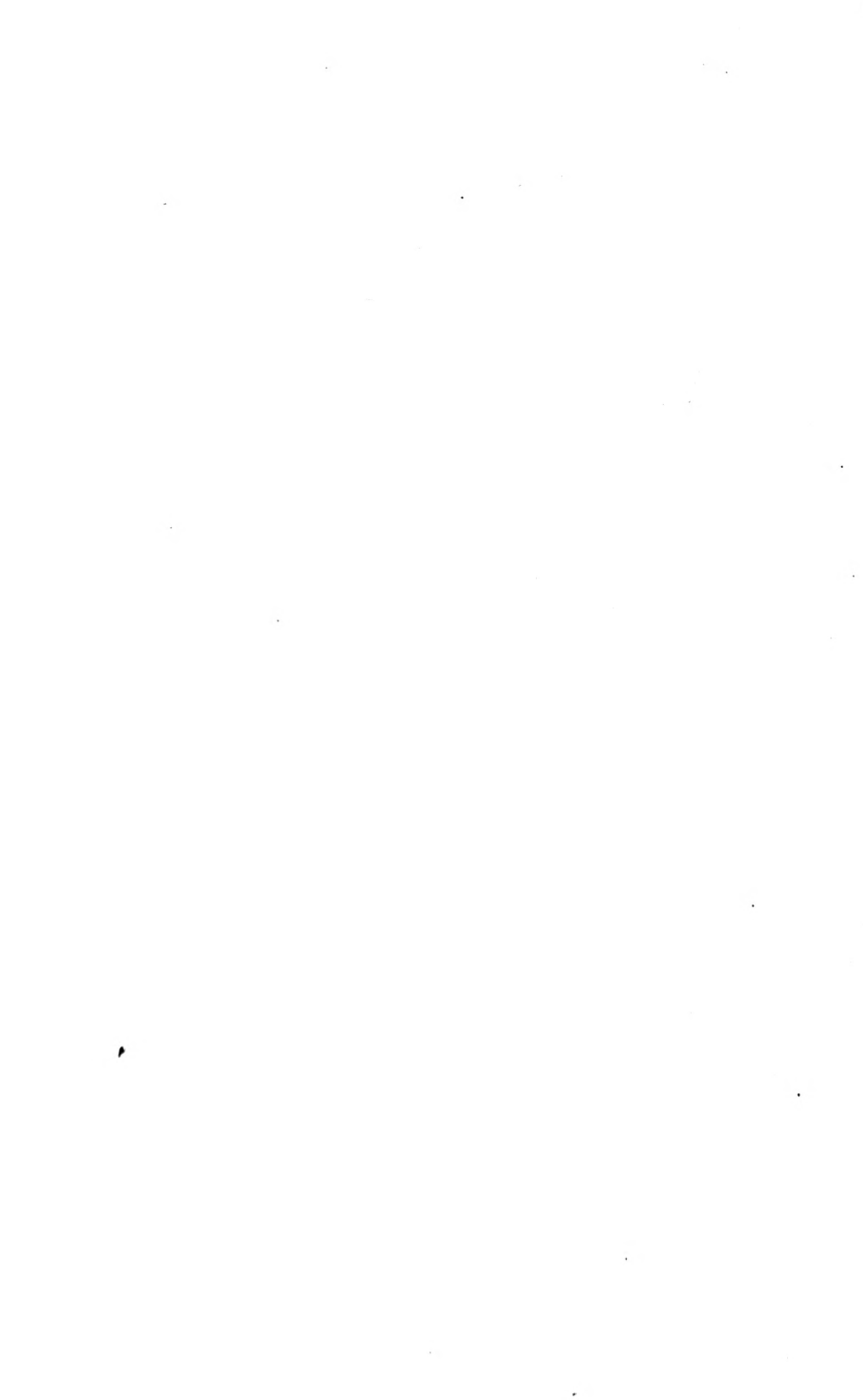
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## VOLUME CHANGES OF THE HEART

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The stroke volume of the heart is probably both for physiological and clinical purposes the most important quantitative function of the whole body. It is much more important than the exact amount of arterial pressure, for the amplitude of the heart's volume change at each beat multiplied by the pulse rate gives the volume of arterial blood supplied to the entire body. Indeed it is particularly from this aspect that at the present time the problem is assuming an importance which is becoming imperative in relation to questions of the oxygen supply of the body, the blood gases, and the coördination of the circulation with respiration during muscular work (Bainbridge), in conditions of asphyxia, and in disease.

Three hundred years ago Harvey solved qualitatively the problem of the volume changes of the heart when he showed that the ventricles are dilated during diastole by the blood returning through the veins. As he saw it, this blood is forced by the contraction of the auricles into the ventricles, and is in turn discharged by them during systole. Harvey scarcely attempted, however, except in the roughest fashion, to determine in quantitative terms the exact volume of blood discharged at each beat. In spite of many investigations devoted to this end in modern times, our textbooks still contain merely qualitative information; and this problem is only now approaching quantitative solution. We do indeed know much regarding the mechanism of the heart, but the coördination and correlation of this organ's behavior with all the other functions of the organism acting as a whole, are still a matter of gaps, guesses, and possibly error.

By analogy with respiration it might be supposed that the heart's tidal volume at a beat is a widely variable quantity. Certainly any one who compares his own sensations when the heart is beating quietly, and when it is pounding in his chest after vigorous muscular work, such as a run, gains this impression. Sketched in its main outlines, respiration behaves as follows: The rate varies from about 15 per minute at rest, up to 40 or more per minute during exercise. Its amplitude varies from 300 or 500 cc. up to four or five times as large volumes of tidal air. In an athlete with increasing exercise it is usually the amplitude of the breaths which alone increases at first; the rate accelerating only after a maximal or nearly maximal amplitude is reached. Within wide limits also the volume of air breathed is such that the oxygen deficit and  $\text{CO}_2$  content of the expired air are each about 4 per cent; indicating that in general the total volume of breathing is directly, if roughly, proportional to the oxygen consumption and energy expenditure of the body. This proportionality holds even more exactly of the pulmonary ventilation, for its variations consist, as Haldane and Priestley showed, of variations in the amount of alveolar air of nearly uniform  $\text{CO}_2$  content replaced by fresh air at each breath.

On comparing the heart with this behavior of the respiratory apparatus we find that the heart likewise varies its rate two- or threefold, from about 70 per minute up to 150 per minute or more; but, unlike respiration, its rate rises even with slight exertion, and increases progressively with each increase of muscular effort. How then does its amplitude of beat vary? According to the all or nothing law each beat is maximal under the conditions prevailing. But does the law apply to this matter, and what are the conditions?

**TWO CONTRASTING THEORIES.** Progress in regard to this problem will be most assisted if we first formulate the two extreme alternative conceptions of the heart's behavior, and show the underlying principles of the variations in the rate of circulation of the blood according to each conception. We may then compare these conceptions with the evidence which the literature affords. It must not be assumed, however, that one conception necessarily excludes the other, for up to a certain point the heart may behave according to one, and beyond that point according to the other, or in some intermediate manner; and during extreme muscular exertion yet another and more or less abnormal behavior may occur. The range of cardiac activity which we shall consider is that lying between bodily rest with an oxygen consumption of 300 cc. per minute or less, and a pulse of 70 or less, up to an exertion such as

can be sustained by a vigorous man for a considerable time and involving an oxygen consumption of about 2,000 cc. per minute and a pulse rate of about 150 per minute.

The first and commonly held conception of the circulation may be termed the theory of variable amplitude of heart beat. It is based on the assumption that the circulation rate—the volume of blood pumped into the aorta per minute—is proportional to the oxygen consumption of the whole body; the circulation would thus in general be analogous to respiration in its behavior. As the oxygen consumption of the body may increase 800 or 1000 per cent over the resting value, while the rate of heart beat increases only about 150 or 200 per cent, that is, 60 beats per minute during rest and 150 to 180 during intense exertion, it is evident that this conception necessarily involves the assumption that the amplitude of the heart beat, its systolic discharge or stroke volume, is variable. In this respect also the heart's behavior would be like that of breathing. The result of this condition would be that, alike at rest and during work, the average venous blood returning to the right heart and mixed in its chambers would contain a nearly unvarying amount of oxygen, and of course, therefore, an unvarying deficit below that in the arterial blood. With this uniform "oxygen utilization," the partial pressure of oxygen in the tissues would also be (presumably) unvarying and as high during work as during rest; an obvious advantage.

The second and alternative conception is that of a nearly uniform maximal heart beat. According to it, the heart action is essentially unlike the respiratory movements of the chest, and there is a varying relation, instead of direct proportionality, between the body's oxygen consumption, or energy expenditure, and the circulation rate. The result would be that during rest the mixed venous blood in the right heart would contain only a little less oxygen than that in the left; during heavy work, on the contrary, the amount of oxygen utilized out of unit volume of blood in its passage through the tissues would render the (average mixed) venous blood far lower in oxygen than the arterial. The partial pressure of oxygen in the tissues during work might be lowered correspondingly. (But see p. 180.)

In the first conception the reserve of the circulation, its factor of safety, is almost wholly in the heart itself, in its variations both of stroke volume and rapidity of beat. In the second conception, the heart contributes only its ability for variation of rate, but not of amplitude, to the factor of safety and to the flexibility of the adjustment of the circulation to the body's demands. The remainder of the margin of

safety, in respect to the utilization of oxygen from the blood by the tissues and especially, the working muscles, amounting to about ten times the resting value, lies in the fact that the blood contains from three to five times as much hemoglobin as a healthy man utilizes for oxygen transportation during rest. Thus if the heart rate rises to three-fold and the oxygen utilization to three and a third, we have ( $3 \times 3.33 = 10$ ) a tenfold variation, even with a uniform stroke volume. The latter conception has thus two considerations to support its probability: one physiological, the other pathological. It explains easily why the corpuscles and hemoglobin are maintained at four times the amount for which there is use during rest; and it explains why an anemic person is easily out of breath even on slight exertion, which according to the first theory should not be the case.

For the sake of still greater sharpness of contrast, let us put these conceptions into figures and thus compare in detail the conditions involved. Suppose a man of strong physique (such as Douglas, the subject of the most valuable experiment in the recent paper of Douglas and Haldane, whose data are here rearranged and generalized) weighing 66 kilos, who consumes 250 cc. of oxygen at rest with a pulse of 60, and 2,000 cc. of oxygen per minute with a pulse rate of 150 when working on a bicycle ergometer. His oxygen pulse, a term introduced by Henderson and Prince (1914) to express the oxygen consumption divided by the pulse rate, or the amount of oxygen which the heart forwards to the tissues at each beat, is 4.16 cc. during rest and 13.3 during work. How would the circulation transport the greater amount of oxygen during work according to the two conceptions? Suppose that, in accord with the first conception, the blood during bodily rest loses 6 volumes per cent of oxygen on the average in passing through the tissues of the body, and that the same rate of oxygen utilization is maintained during work; in other words, that alike in work and rest the blood in the right heart contains 6 cc. less of oxygen per 100 cc. of blood than that in the left heart. Evidently then the rate of circulation, or total blood discharged into the aorta per minute, must vary in direct proportion to the oxygen consumption. The blood flow during bodily rest would therefore be 4166 cc. per minute, and that during work 33,333 cc. Each divided by the pulse rate gives the stroke volume of the left ventricle: 69.4 cc. of blood for rest and 222 cc. for work. These relations are shown in table I A.

On the other hand suppose that with the same data of oxygen consumption, pulse rate and oxygen pulse, we should find that the oxygen

utilization during rest is only 3.33 volumes per cent, while during work it is 10.7: a relation which would still leave 6 or 7 volumes per cent of oxygen unutilized in the venous blood, since the arterial blood contains about 18 cc. of oxygen per 100 cc. of blood. Then the circulation in rest would be 7,500 cc. of blood per minute and during work, 18,700 cc.; and alike during rest and work the stroke volume would be 125 cc. These relations are shown in table 1 B.

TABLE 1

*Illustrating two contrasting conceptions of stroke volume and circulation rate*

Subject, man, 66 kilos

THEORY	OXY- GEN CON- SUMP- TION	PULSE RATE	OXY- GEN PULSE	OXY- GEN UTILI- ZATION	CIRC- ULATION RATE	SYS- TOLIC DIS- CHARGE	CONDI- TION OF SUB- JECT	POSTULATES
	cc. per minute	per minute	cc. per beat	volumes per cent	cc. per minute	cc.		
A	250	60	4.16	6	4,166	69	Rest	Variable amplitude of beat and stroke volume, with uniform relation of circulation rate to oxygen consumption
	2000	150	13.3	6	33,333	222	Work	
B	250	60	4.16	3.33	7,500	125	Rest	Uniform maximal beat with varying relation of circulation rate to oxygen consumption
	2000	150	13.3	10.7	18,700	125	Work	

The evidence regarding these alternative conceptions lies in a number of quite separate fields, which we shall now survey.

**LIMITING ANATOMICAL CONDITIONS.** It is highly probable that during health even the largest systolic contractions never entirely empty the ventricles. Both plethysmographic observations on the heart (the volume curve) and x-ray pictures support the older evidence (as reviewed by Tigerstedt, 1893 and 1907) that at the end of systole a considerable volume of blood remains in the ventricles; at least a quarter, perhaps a third, or even a half as much as the systole discharges into the arteries. The systolic size of the heart depends both on tonus and on the amplitude of contraction. The diastolic size depends upon tonus and amplitude of relaxation, amount of venous pressure and inflow, and finally upon the pericardial sack.

The heart is not free to expand indefinitely, for one need only inspect the heart of an animal killed quickly by asphyxia to see the chambers so distended that the pericardial sack is stretched tight. Barnard (with Leonard Hill) showed that the pericardium is practically inextensible, and that it can bear a pressure of  $1\frac{1}{4}$  to  $1\frac{3}{4}$  atmospheres. He found that the dead heart, in which tonus is completely absent, can expand to twice the size upon opening the pericardium that it can with the pericardium intact. Evans and Matsuoka found an increased gaseous exchange in the heart, indicating increased work, after opening the pericardium. Kuno (with Starling) found in the heart-lung preparation that opening the pericardium had a very distinct effect in increasing the amount of blood pumped by the heart when the venous inflow was large. In particular the pericardium seemed to protect the right heart from over distention. His opinion "that the pericardium exercises under every condition of venous inflow a certain amount of resistance to the diastolic expansion of the heart, which increases with any increase in the heart volume," seems however, very improbable. His opinion that "the existence of the pericardium is necessary for the unimpaired working of the heart in normal life" is negatived by the investigations of Yamada, who (working in association with Kuno later) found that animals, in which the pericardium had been cut open aseptically, the chest closed again, and the subjects restored to normal health, were able to make vigorous physical exertion with no injury to the heart. He offers the very probable explanation that during increased rapidity of beat, the corresponding increase of tonus prevents the ventricles from dilating so far.

It seems probable from these observations and from the volume curve (see below), that if the pericardium in a normal man or animal limits the extent of dilatation at all, this occurs only during a period of very slow beats and very low tonus during sleep: it is not protective against cardiac strain. All the ordinary volume changes of the heart probably fall short of either of the limits of diastolic distention and systolic contraction thus defined, and depend upon the behavior of the heart itself under the control of nervous, chemical and mechanical conditions, the latter chiefly venous pressure. It is, however, of some interest to know the possible maximal volume change in man between these limits.

To determine this point Henderson and Prince (1914) made the following observations: On six cadavers at autopsy, 3 men and 3 women, water was run into the pericardial sack, through a funnel, rubber tube

and hollow needle, up to maximal distention, and the amount was measured. The volumes of blood obtained from the heart's chambers later in the autopsy were also measured. The sum of the two measurements gave figures of 650, 700 and 620 cc. respectively in the men; and in the women, 633, 430 and 350 cc. As two of the men were young and of good muscular development, 700 cc. represents a fair estimate of the normal maximum volume. Bohr had previously made similar determinations on the pericardial sack of the horse and found 2 liters for each side of the heart in a horse of 500 kgm. He concluded that even if the heart passed from the greatest distention which the sack would allow down to complete emptiness at each stroke, the systolic discharges would be insufficient to allow an oxygen transportation equal to the maximum oxygen consumption of the animal during hard work found by Zuntz and Hagemann; Bohr (with Henriques) was thus trying to show a considerable consumption of oxygen in the lungs, a conception no longer supported by anyone. Zuntz (1911 and 1912) in answering Bohr and Pütter had the matter reinvestigated by Müller (1911), who found the total pericardial volume in the horse 0.78 to 0.93 liter per 100 kgm., virtually agreeing with Bohr's figures. Some observations on dogs and rats in this laboratory (unpublished) have yielded figures of the same order in cubic centimeters per kilo.

If we use the figure 700 cc. for the pericardial volume in man and assume that each auricle has half the capacity of its ventricle, we have 233 cc. as the extreme volume which the sack would allow each ventricle to expand and contract. According to the theory of variable systolic discharges, as shown in table 1 A, such a man as is there considered must have a stroke volume, if the first theory is correct, of 222 cc. when the amount of body exertion consumes only 2,000 cc. of oxygen per minute. As a vigorous man may do far more work than this and consume a much larger amount of oxygen per minute, the first theory in its strictest form would seem to require that for an oxygen consumption of 3,000 cc. per minute, the ventricles should be distended to the full amount that the pericardial sack would allow during diastole, and contract to the obliteration of the chambers during systole; and even then the heart would fall short of accomplishing its task. Hiffelsheim found, by filling the ventricles with wax, a capacity of only 156 cc. for the left ventricle of man, and the right slightly larger. Keller has made similar measurements.

To sum up, the pericardial volume for all the animals and men observed is about 3.5 to 5.0 cc. per kilo body weight for each side of the

heart. It will be shown below that the maximum stroke volume of each ventricle is probably about 1.5 to 2.0 cc. per kilo for all vigorous mammals.

EQUATIONS OF OXYGEN CONSUMPTION AND BLOOD CIRCULATION. In considering the investigations to be reviewed below, it is essential to keep particularly in mind the inverse relation of the stroke volume of the heart to the amount of oxygen (in volumes per cent) utilized from the blood. The oxygen pulse (the oxygen consumption divided by the pulse rate) is easily calculable for every heart rate and degree of muscular exertion in man. If then, for example, the oxygen pulse,  $S_o$ , is 6 cc., and the oxygen utilization,  $U_o$ , is 4 volumes per cent, the stroke volume,  $S$ , is necessarily 150 cc.; or stated in general terms, the solution of our problem by the respiratory or blood gas method (as shown hereafter) turns upon determining the oxygen utilization and then calculating the stroke volume by means of the expression:

$$S = \frac{S_o \times 100}{U_o}$$

Evidently if  $S$  is a constant for the individual, the relation of  $U_o$  to  $S_o$  is also constant.

This expression is a derivative of the equation generally used in this connection in which  $M$  is the minute volume or total circulation rate,  $O$  is the oxygen consumption of the body per minute, and  $U_o$  the oxygen utilization in volumes per cent, and which is as follows:

$$\frac{M}{O} = \frac{100}{U_o}$$

The oxygen pulse from such data as are now available appears to be nearly uniform (about 3.5 cc. to 5.0 cc. for a 60 or 70 kilo man) at rates below that of sitting rest (about 70 beats per minute), rises rapidly up to heart rates of 100 to 120 per minute (12 to 15 cc. oxygen per beat) and then less and less rapidly with further acceleration of the heart rate. There are probably marked differences between athletic and sedentary individuals, and considerable variations in the individual depending upon the momentary degree of nervous excitement, and upon whether large muscle masses are worked moderately or small masses vigorously with the same oxygen consumption.

To express our problem in its simple terms: The theory of a nearly uniform maximal heart beat, as large during rest as during work, is

tenable only if it can be shown that in vigorous normal men and animals the oxygen utilization during rest is quite small, 3 to 5 volumes per cent or less; but if this condition can be demonstrated this theory is thereby virtually established. On the other hand, the finding of a stroke volume of only 0.3 cc., or less, per kilo at a normal venous pressure, as in many experiments by means of the stromuhr and on the heart lung preparation, would necessitate the acceptance of the theory of a considerable oxygen consumption in the lungs: a point on which Tigerstedt (1905) has justified the correctness of Bohr's argument.

In spite of many opposing witnesses, evidence is accumulating that the condition of a very small oxygen utilization during rest is fulfilled in vigorous men.

**OBSERVATIONS BY RESPIRATORY METHODS.** The classic work of this type is that of Zuntz and Hagemann on the metabolism of the horse during rest and work. In the chapter on the work of the heart in that animal, using the methods first suggested by Fick (1870 and 1886), they estimated the circulation rate from the following data: *a*, amount of oxygen absorbed, or CO<sub>2</sub> exhaled by the lungs; *b*, the amounts of oxygen and CO<sub>2</sub> in blood drawn from an artery; and *c*, the amounts of these gases in blood drawn from a sound inserted through the jugular vein into the right heart. The differences in respect to the oxygen and CO<sub>2</sub> in the arterial and mixed venous blood divided into the total oxygen or CO<sub>2</sub> of respiration per minute gave the circulation rate. As the average figures for the best nine or ten experiments on animals of the average weight of 347.6 kgm. at rest, they found differences between the arterial and venous blood of 7.44 volumes per cent oxygen and 7.16 volumes per cent CO<sub>2</sub>; and for work differences of 9.34 for oxygen and 8.17 for CO<sub>2</sub>. Arterial blood in the rather poor horses used contained 14 or 15 volumes per cent of oxygen, but in vigorous animals was estimated by Zuntz to contain 19.5. As the oxygen consumptions were 2080 cc. at rest and 5007 cc. during work, the blood pumped out of the left heart per minute figured out to 29,155 cc. during rest and 53,034 cc. during work. No counts of the pulse rate are given in their protocols, but the rates assumed were 40 beats per minute in rest and 55 during work. The stroke volumes calculate to 729 cc. during rest and 964 cc. during work, or 2.09 cc. per kilo body weight for rest, and 2.77 cc. per kilo for work. (If 70 beats per minute were assumed during work, the systolic discharge would have been the same as during rest.) The attention of the investigators was fixed, not primarily upon the blood flow, but upon the work of the heart (the circulation rate multi-

plied by the arterial pressure), and for this purpose the actual pulse rates were unimportant.

The lack of precise counts of the pulse deprives this work to a considerable extent of the evidential value sometimes assigned to it in favor of the theory, which Zuntz always defended,<sup>1</sup> of a variable amplitude of heart beat, increasing during physical work. The stroke volume determined during rest (2.09 cc. per kilo) is, however, not subject to this criticism. In one experiment of intense work, in which the animal consumed 22,942 cc. of oxygen per minute, Zuntz and Hagemann estimated that 245.5 liters of blood were pumped in 70 beats of the heart, or 3.5 liters per beat by each ventricle, or about 10 cc. per beat per kilo body weight. Here surely there must be some gross error either in the original data or in the method of calculation. Bohr made very effective use of this experiment by pointing out that a stroke volume of 3.5 liters in a horse was an anatomical impossibility, and that the data were comprehensible only on the basis of a large consumption of oxygen in the lungs: a contention which Zuntz rejected, but which on account of this experiment he had some difficulty in explaining away. Bohr held it to be extremely improbable that the heart is capable of doubling its rate of beat and its stroke volume simultaneously.

While inclining in the main toward the theory of variable heart beat, Zuntz and Hagemann nevertheless say expressly that "in the working animal the circulating blood is better utilized than during rest, in that per unit blood more oxygen is withdrawn and more CO<sub>2</sub> added in the tissues."

Grehant and Quinquaud had already carried out similar experiments on dogs, but based their conclusions on determinations of the excess of CO<sub>2</sub> in the venous blood over that in the arterial blood, amounting in six experiments to 11.3, 8.5, 4.6, 4.0, 4.2, 5.5 volumes per cent of CO<sub>2</sub>. They report no experiments during muscular activity and no counts of the pulse; but merely give the calculated circulation rates, varying from 512 to 2611 cc. for dogs from 7 to 18 kilos. If we assume that the smaller figures are for the smaller dogs and the larger for the larger dogs, and further that the pulse was always 100, we find stroke volumes of 0.73 and 1.45 cc. per kilo respectively.

<sup>1</sup> At the International Physiological Congress in 1910, the reviewer presented evidence for a uniform stroke volume from volume curves, and had the honor of a brief debate with Professor Zuntz over this matter,—a debate greatly hampered by linguistic difficulties—in which Professor Zuntz strongly objected to the view presented.

Loewy and von Schrötter attempted the solution of this problem on man. Following the lead of Pflüger and his pupils, Wolffberg and Nussbaum, in the use of a similar technique on animals they inserted a catheter or sound through the mouth and trachea in such a way as to close off the right bronchus or one of its larger branches. Air samples could be drawn at intervals from the part of the lung thus cut off from external ventilation, and it was found that the gases which it contained quickly assumed a uniform composition, corresponding to the tensions of the gases in the venous blood coming to the lungs. By applying the oxygen tension thus found to the oxygen dissociation curve of blood, a value for the oxygen content of the venous blood was obtained. A corresponding value for the arterial blood was obtained from the percentage of hemoglobin in the blood and the oxygen in the air in the ventilated lung, so that the oxygen utilization was calculable. It was found that the  $\text{CO}_2$  tension of the venous blood was surprisingly low, only 6 per cent of an atmosphere. The oxygen utilization during rest was 6.5 cc. per 100 of blood. For a 60 kilo man therefore, during rest, the circulation rate was figured at 3.85 liters per minute. They concluded that the stroke volume is subject to wide variations, but is on the average 55 cc. for a 66 kilo man, or 0.83 cc. per kilo body weight.

Although Loewy and v. Schrötter discuss the effect of work upon the circulation, their experiments on this topic were few and the amount of work so small, as judged by the oxygen consumption, as really to throw little light on the subject.

Plesch, working in part in Zuntz's laboratory, greatly simplified the technique of estimating the venous blood gases in man from respiratory data. His procedure in principle consisted in breathing back and forth for 15 to 25 seconds into a small rubber bag so that the air in the bag came to be of the same composition as that in the lungs and was thus in gaseous equilibrium with the venous blood. The  $\text{CO}_2$  tension was thus easily determined. To obtain the oxygen tension it was necessary that the bag at the outset should be filled with nitrogen. In this way without risk or inconvenience to the subject, the gas tensions of the venous blood were obtained and the oxygen content calculated. Although Plesch does not emphasize the point, his data show that the  $\text{CO}_2$  partial pressure of the venous blood is, as Loewy and v. Schrötter had also found, only a little above that of the arterial blood. The oxygen utilization in 5 normal people at rest was 7.4, 4.3, 7.15, 5.74 and 4.26 cc. per 100 cc. of blood. In a healthy resting man of 68 kilos, he estimates the minute volume of the circulation at 4,300 cc. and the

stroke volume at 59 cc. or 0.86 cc. per kilo body weight. He holds that the circulation rate depends upon the combustion in the tissues, but that the increase is not proportional to increase of oxygen consumption during work. He considers that the blood never loses more than two-fifths of its oxygen in passing through the tissues. He estimates the highest possible minute volume during the most violent work at 47 liters of blood and the stroke volume, in a man working hard, at 240 cc. But he did no experiments and made no measurements during work.

Bornstein also attempted to estimate the volume of the blood stream in man from respiratory data. The lungs were rapidly washed out with oxygen and the subject thereupon breathed through a potash cartilage into a bag filled with oxygen. From the rate at which the nitrogen dissolved in the blood was given off to this atmosphere of oxygen, he calculated the volume of blood passing through the lungs. His data figure out to a minute volume of 6 liters for rest, but during work would indicate, as Krogh justly comments, the impossible minute volume of 60 liters.

Markoff, Müller and Zuntz utilized the breathing of nitrous oxide for the purpose of estimating the circulation rate in man. In one determination they found the stroke volume of the heart 96.7 cc., which they regarded as too large. In another determination on the same subject they found 43.9 cc.

Krogh and Lindhard (1912) developed the nitrous oxide method independently. In their procedure the gas (10 to 25 per cent nitrous oxide in air enriched with oxygen) is held in the lungs for a period of less than the time of a full circulation. At the beginning and end of this period, which must be accurately measured, an alveolar air sample is obtained and analyzed particularly for nitrous oxide and for oxygen. The total volume of gas in the lungs is also estimated from previous respiratory data, the oxygen consumption of the body is determined either before or after the nitrous oxide experiment, and the pulse is counted. From these data and the coefficient of distribution of nitrous oxide between the air and blood in the lungs, the blood flow per minute through the lungs is calculated. There are thus a number of factors to be estimated and combined, and the observational errors in alveolar air methods, as Krogh and Lindhard (1914 and 1917) have themselves pointed out in a critique of the work of some other investigators, may be considerable. It is to be expected, therefore, that in the combination of the various factors during a calculation, the observational errors may

in some cases neutralize one another, and in others may be summated in the results. When we examine these results, we find exactly the sort of variations to be expected on these grounds. The investigators themselves seem, however, to assume that the widely varying systolic discharges and circulation rates, which they obtain even in the same subject under identical conditions on different days, are correct expressions of physiological variations in heart action, instead of mere experimental errors and variations. Particularly in a later paper (in Pflüger's *Archiv*, clxi) in which Lindhard (1915) reports numerous observations, the variations between successive determinations are very large. Thus for example in table XIc, the subject J. J. on February 22nd, during work involving the consumption of 870 cc. of oxygen and a pulse rate of 92, showed a stroke volume of 160 cc., and 4 days later with the same pulse rate and the same oxygen consumption, the stroke volume was only 88 cc. Again in table IX, the subject J. L. on June 19th with an oxygen consumption of 225 cc. and a pulse of 64 showed a stroke volume of 71 cc., but on November 26th, with an oxygen consumption practically the same and the same pulse rate, the stroke volume found was only 49 cc. These are extreme examples, but to a considerable degree variations of this type run through all the data reported by Lindhard.

Such variations would not, however, necessarily invalidate the conclusions, for Lindhard (1916) has obtained so large a mass of observational material that he is able to treat it statistically. Statistical treatment does not, however, eliminate a fundamental disturbing element; and such an element in this work lies in a correction which is applied to all of the blood flow measurements. This correction depends upon the fact that during the experiment itself, while nitrous oxide is being held in the lungs, the oxygen consumption apparently differs markedly from that during the fore and after period; the difference amounting in some cases to an increased rate of absorption of 60, 80 or even 100 per cent or more. The figures finally calculated for blood flow and stroke volume are therefore corrected, that is, reduced, by corresponding amounts. The extent of the correction in some cases may be illustrated (from table V) by the subject J. J. on whom between the 8th and 15th of October were obtained the following figures for the minute volume of the circulation (in liters). The unreduced figures as calculated directly from the observations are here placed above the "corrected" figures:

Observed	8.8	10.3	9.5	9.7	7.7	7.9	9.0	7.0
Corrected	7.4	8.55	7.9	6.5	5.6	5.7	4.2	3.0

Some of these, notably the last two, are indeed enormous corrections and render the "corrected" figures much more variable than those directly obtained, which latter are, it now appears, also probably much nearer the true values.

This correction is in many cases inconsiderable during experiments on work, and Lindhard's measurements of the minute volume of the circulation and the systolic discharge during work are, on the average, aside from the experimental variations, probably of the correct order of magnitude. The determinations during rest, in the light of later work to be reviewed below, are probably only 50 to 60 per cent of the correct value. It is true that the correction itself appears at first sight logical; but it requires us to assume that nitrous oxide exerts no pharmacological effect, and also to believe that during the holding of the breath after 3 full breaths the circulation may be accelerated even up to 100 per cent. In fact, however, little is known of the pharmacological action of nitrous oxide; and the radial and carotid pulse give to one's finger no indication of such augmented beats during such breathing and pause; as the reader may immediately convince himself. It may be mentioned also that in connection with the Pike's Peak expedition (Douglas, Haldane, Henderson and Schneider, prior to the publication of the work of Markoff, Müller and Zuntz and that of Krogh and Lindhard) the reviewer had independently attempted to utilize nitrous oxide to determine the circulation rate. In these experiments it was found that marked functional disturbances of an obscure character in respect to the absorption of oxygen occurred and that the results obtained were subjected to so much doubt and error that the method was not developed.

Douglas and Haldane have justly pointed out that one of the probable sources of error in Krogh and Lindhard's method lies in the first alveolar sample giving an oxygen percentage higher than existed at the moment in the deep alveolar air. Lindhard (1922) in his latest paper seems to concede at least in part the validity of the objection.

The general conclusions reached by Krogh and Lindhard and by Lindhard in later work are that "the blood flow during rest varies between very wide limits (from 2.8 to 8.7 liters per minute) depending upon the variable supply of venous blood to the right heart." When a series of consecutive determinations is made "a remarkable constancy is, however, generally observed." (But compare the figures above quoted.) "During muscular work the rapidity of the circulation is greatly increased, the maximum observed being 21.6 liters per minute.

Oxygen utilization during rest is expressible by coefficients varying between 0.28 and 0.60 rising to 0.73 during muscular work." "The stroke volume may fall as low as 26 cc. during rest." Lindhard concludes that "during rest the minute volume is a function of the respiratory metabolism, a metabolism of 200 cc. of oxygen corresponding to a blood flow of 3.5 to 4 liters." The coefficient of utilization of oxygen from the blood is 0.3, a little higher for women and a little lower for men. The systolic discharge of the heart is in all cases a secondary function determined by the two independent variables, minute volume and pulse frequency. During muscular work the minute volume may increase sixfold while the metabolism increases tenfold, and the oxygen utilization may then reach a coefficient of 0.795, corresponding to 14.85 cc. of oxygen per 100 cc. of blood.

In spite of the technical defects of the nitrous oxide method, it is probable that the average of the observations for the circulation rate and stroke volume during muscular work is of the correct order of magnitude. It may be pointed out also that if Krogh and Lindhard had not felt compelled to reduce their values by the correction above mentioned, the average of their determinations on rest would also be in accord with later and more accurate estimates of the circulation rate and stroke volume for this condition. Indeed it is noteworthy that in some experiments they found a figure (103 cc.) for the stroke volume during rest which as they point out "falls very little short of the largest stroke (116 cc. recorded, or 127 cc. assumed) during work."

The nitrous oxide method has been used also by Fridericia (1916, 1918), Liljestrand, Boothby, and Means and Newburgh, with results generally similar to those above quoted. They find stroke volumes of 1.5 to 2.0 cc. per kilo body weight during work, but only about half or two-thirds as large during rest, and subject to wide variations under identical conditions.

Means and Newburgh have also attempted to calculate the circulation rate and stroke volume from the oxygen unsaturation of blood drawn from a vein in the arm. Assuming the arterial blood to be 95 per cent saturated, the coefficient of oxygen utilization was thus found in one observation 0.29 and in another 0.49, or about 6 and 9 volumes per cent. From these data the stroke volumes would figure to 75 and 34 cc. Similar figures could be based on analyses of the arterial blood and blood from an arm vein of normal men contained in papers by other recent investigators. Thus Lundsgaard's (1919) data show an oxygen utilization of 5.5 volumes per cent; those of Stadie 4.9 to 6.3;

and those of Harrop 2.5 to 8.3. In dogs Doisy and Beckmann report finding differences in the oxygen content of blood in the femoral artery and vein varying in 17 subjects from 2.1 to 18.1, average 6.1 volumes per cent. Uyeno and Doi find similar variations in the cat. Lundsgaard and Möller find that after vigorous exercise (stair climbing) the oxygen content of the blood from a vein in a man's arm is much decreased.

Unfortunately the oxygen utilization in a vein draining merely muscles and skin can afford no reliable indication of the oxygen utilization for the whole blood stream. Leonard Hill and Nabarro have shown that the coefficient of oxygen utilization for blood passing through muscles is 0.7 or 0.8, which would be 12.5 to 14.5 volumes per cent for human blood, while that for blood from the brain is only 0.2 or 0.3. Barcroft (1920) also notes the striking contrast between the color of the bright blood from the kidneys and the dark color of that returning from the hind legs as seen in the rabbit at the junction of the renal veins and the vena cava. In view of such observations as these, it seems quite possible, although paradoxical, that although the oxygen utilization for the whole blood stream is very much higher during muscular work than during rest, each tissue or organ has a nearly unaltered percentage utilization. It is the greater quantity of blood flowing through the muscles during work (Chauveau and Kaufmann) which causes the higher utilization for the whole blood stream. During progressive asphyxia practically the whole oxygen load of the blood may be utilized (Greene and Greene).

Boothby, Fridericia, and Liljestrand and Lindhard have also used the Fick principle for determining the blood flow in man, with results similar to those from the nitrous oxide method.

The most important advance in this field in recent years has come from the work of Christiansen, Douglas and Haldane. They demonstrated the mutual interaction of oxygen and  $\text{CO}_2$  in the blood and were the first to work out the dissociation curve of  $\text{CO}_2$  in blood: technical matters which are fundamental for valid work in application of the Fick principle to man. They also began the work on the measurement of the blood flow which has now been completed by Douglas and Haldane (1922). These investigators show for the first time on man that, at least in vigorous subjects, the stroke volume of the heart may be a quantity nearly uniform in work and rest. They say "In Douglas the circulation rate was nearly in proportion to the pulse rate. This corresponds to the conclusion reached in 1906 by Yandell Henderson from plethysmograph records of the heart beats in dogs. It has been inferred that Henderson's conclusions must have been mistaken, since

assuming as has hitherto been done that the mixed venous blood loses at least a third of its oxygen during rest, an increase to ten times or more in the metabolism during work could not possibly be brought about without a very large increase in the output per beat. The figures for Douglas show, however, that no increased output per beat, but only the actual increase of about three times in the pulse rate was needed for an increase in metabolism to ten times the basal rate. In none of our subjects was the circulation rate during work increased in even approximately direct proportion to the increase in metabolism, until the metabolism had been greatly raised."

The essential feature of these observations is that in such a subject as Douglas (66 kilos) the stroke volume (125 cc., or 1.9 cc. per kilo body weight) and the circulation rate (7,500 cc.) are so large that during rest the coefficient of oxygen utilization is only about 20 per cent, or a difference of only 3.2 volumes per cent in the oxygen content of the blood in the two sides of the heart. Thus when work is done the increased pulse rate (up to threefold) and the increased oxygen utilization from the blood (up to three- or fourfold) together provide for a tenfold augmentation of the energy expenditure and respiratory metabolism. (See table 1, page 169.)

On the whole it seems probable that a low coefficient of oxygen utilization during rest and a stroke volume as large during rest as during work are characteristics of vigorous athletic men. A stroke volume of 1.8 to 2 cc. per kilo body weight during work is quite in harmony with other investigators, but it is twice as large as has generally been believed heretofore to occur during rest. The idea involved may be illustrated by comparing the conditions in so unenergetic an animal as the domesticated goat, with the conditions in dogs. Thus Barcroft and his collaborators (1920) have found that in the goat with 66 per cent hemoglobin (Haldane's scale) the difference in the oxygen content on the two sides of the heart is 4 to 7 cc. of oxygen per 100 of blood. The minute volume per kilo works out to 133 cc.

On the other hand in experiments (as yet unpublished) of a similar character, performed in this laboratory by Haggard and the writer upon dogs, the blood in the left heart contained 20 volumes per cent of oxygen and 39 of CO<sub>2</sub>, while that in the right heart contained 17 oxygen and 41 CO<sub>2</sub>: difference, 3 volumes per cent oxygen, and 2 CO<sub>2</sub>. This low coefficient of utilization indicates a stroke volume of 1.9 cc. per kilo body weight; the heart rate was 130 per minute. In another experiment the arterial gases were 16.5 oxygen, 47.5 CO<sub>2</sub>, while the venous

gases were oxygen 12,  $\text{CO}_2$  51; difference 4.5 oxygen and 3.5  $\text{CO}_2$ . The stroke volume figures therefore to 1.58 cc. per kilo body weight at a pulse rate of 126 per minute. In a third dog the difference between the blood gases in the right and left hearts was 3.5 oxygen and 1.5  $\text{CO}_2$ . Evidently goats have only a small reserve in respect to oxygen utilization upon which they can draw during exertion, while dogs have an enormous reserve, 400 or 500 per cent. The capacity for vigorous exertion would thus be 2 or 3 times as large in dogs as in goats.

That vigorous men are in this respect comparable to dogs rather than to goats in the smallness of the difference in the amount of  $\text{CO}_2$  in arterial and venous blood during rest has been found by Henderson and Prince (1917) in a number of determinations (still in progress, Henderson, 1922) by a development of the respiratory method. The  $\text{CO}_2$  tension in the alveolar air or (as it were better termed) "arterial pulmonary air" is determined by Haldane's method, while that in the "venous pulmonary air" is found by rebreathing, by Plesch's method air containing about 6 per cent  $\text{CO}_2$  and a high enough oxygen content to nearly saturate the blood with this gas. This gives, not the true venous  $\text{CO}_2$  tension, but that for venous blood which has been saturated with oxygen without loss of  $\text{CO}_2$ . Both the arterial and the virtual venous  $\text{CO}_2$  tensions thus obtained may then be applied to the  $\text{CO}_2$  dissociation curve of fully oxygenated blood (a curve of which the slope is sufficiently uniform, although the absolute level varies individually) to obtain the corresponding figures for the  $\text{CO}_2$  contents of the arterial and mixed venous bloods. The  $\text{CO}_2$  difference (in volumes per cent) multiplied by the inverted respiratory quotient gives the oxygen utilization.

In healthy men at rest the values thus found are seldom more than 3 or 4 volumes per cent of  $\text{CO}_2$  difference in the arterial and venous bloods; and although larger differences are sometimes found, they are generally observational errors rather than true indications of slower circulation. Barcroft, Roughton and Shoji report that the method of Henderson and Prince gives results in close agreement with a more refined method of the same general character employed by them. The  $\text{CO}_2$  differences found by Barcroft and his co-workers on two subjects were 4.0 and 3.3 cc. Redfield, Bock and Meakins also confirm the method of Henderson and Prince and report correspondingly low  $\text{CO}_2$  pressures in the venous blood.

Meakins and Davies (1922) by a method essentially similar to those just mentioned have obtained very concordant and significant results.

On four human subjects they find the stroke volume to be during rest 100, 150, 132 and 96 cc. and on a fifth subject 121 cc. during rest with an oxygen consumption of 217 cc. per minute, and practically the same stroke volume, 122 cc., during exercise on a bicycle and with an oxygen consumption of 1104 cc.

We know that man is a very vigorous animal. It appears from these data that his vigor in great part is based upon the large factor of safety afforded by a low oxygen utilization, with a correspondingly large stroke volume, during rest.

OBSERVATIONS ON THE CIRCULATION TIME. Numerous attempts have been made to estimate the heart action in man from observations on the peripheral circulation. Such methods as the arm plethysmograph (Fick, 1869; Hewlett, 1909), the hand calorimeter (Stewart, 1911), and similar procedures have yielded valuable evidence in their own fields; but they have not contributed materially to knowledge of the stroke volume of the heart and the velocity of the total blood stream.

The attempt to develop a rule by which the product of the pulse rate multiplied by the pulse pressure would serve as an index of the circulation rate in man has given a method of some value for purposes of comparison, but has not thrown much light on absolute quantities. (Dawson, Erlanger and Hooker, Skelton). Nor has it yet proved practicable, except for comparisons, to utilize as an estimate of the heart's activity, the recoil curve of the whole body (Henderson, 1905, Heald and Tucker). This curve records a movement of the body of about 0.1 mm. headward and feetward under the influence of the mass movements of the heart and blood, when the subject lies on a table in delicate suspension. Very little evidence of value on our topic has come from sphygmographic or electrocardiographic methods.

The duration of a circulation, the time required for the blood to complete the round and return to the same point, was first determined by E. Hering. He showed that in mammals of different sizes the circulation rate varies as the body surface. If the total volume of blood in the body is known, and if the velocity at all points and in all organs were equal, this method would be effective. Hering injected an easily recognizable non-poisonous substance, potassium ferrocyanide, into a vein toward the heart, and noted the time until the substance appeared in the blood flowing from another similar vein. The method was also utilized by Volkmann, who found on several species of animals that the stroke volume of the heart was 2.5 cc. per kilo body weight.

By Vierordt the method was refined and yielded much valuable information. He found that in mammals of widely different sizes, goat, dog, man and horse, the circulation time was in all cases between 26 and 28 heart beats. (In determinations of the venous pulmonary air by the Plesch method in this laboratory the second rise of  $\text{CO}_2$ , indicating completion of the shortest way round the circulation, is found to occur in approximately this number of heart beats in man.) This is a striking example of relativity, as the pulse rate in the rabbit is 4 or 5 times as rapid as in the horse, while in animals of intermediate weight the pulse rates correspond with the relative size. For mammals of all sizes, according to Vierordt's results, the stroke volume has the same magnitude in cubic centimeters per kilo body weight; and the circulation rates in mammals of all sizes, in cubic centimeters per minute per kilo body weight, vary therefore as the pulse rates. Vierordt figured the stroke volume at 2.83 cc. per kilo. v. Kries pointed out, however, that as the blood passes through some organs more rapidly than through others, as evidenced by its varying venosity, the stroke volume thus calculated would be too high and the indicated circulation rate too rapid.

Vierordt's conceptions appear to the reviewer much nearer probable truth than those of most later investigators, and combined with other data in this review they suggest the probability of the following laws (modified from Vierordt's statement): 1. The average duration of a circulation through the shortest channels in all vigorous species of mammals at rest is equal to the average time of  $27 \pm 3$  heart beats of the species. 2. In different species of vigorous mammals there is direct proportionality between the blood flow (expressed in cubic centimeters per kilo per minute) the resting pulse rate, the oxygen consumption per kilo, and the surface: weight ratio; all four values for different species are thus proportional to each other. 3. The stroke volumes of the hearts in species of mammals of different sizes vary directly as the body weights for all species of active mammals. In physiology such laws are never better than rough approximations and this formulation is offered not as definitely proved, but as valuable for test and suggestion. The more one studies the literature upon which this review is based, and data which lack of space prevents reproducing here, the stronger becomes belief in the essential correctness of some formulation of the sort here amplified on Vierordt's foundation.

The method of the circulation rate has been used from time to time by later investigators and notably in a modified and improved form by

Stewart (1894, 1921), who injected 1.5 per cent NaCl solution into a vein and determined its appearance in an artery by the change in electrical conductivity of the blood. In a typical experiment 33.5 cc. of salt solution were injected in 12 seconds. When the telephone indicated the arrival of the diluted blood in a branch of the femoral artery, 25 cc. were drawn during the 7th to the 17th second. From this sample the extent to which the blood was diluted was determined and the amount of blood discharged by the left heart was calculated. By this method in dogs values for the stroke volume of from 1.7 cc. in large animals to 3.2 cc. in small animals were found. Stewart, however, concluded that the circulation rate can vary considerably even at a constant pulse rate or may remain the same during a varying pulse rate. He argued that the stroke volume would grow smaller per kilo body weight as the size of the body increased and he therefore estimated the stroke volume of man at about 75 cc.

**DIRECT MEASUREMENT OF BLOOD FLOW IN OPERATED ANIMALS.** There have been many attempts to determine the stroke volume and circulation rate by measurements of the blood flow particularly in dogs and rabbits by such techniques as the stromuhr (R. Tigerstedt, 1891; C. Tigerstedt, Elving and v. Wendt), the plethysmograph for the whole heart (Roy and Adami, Johansson and Tigerstedt), and other related devices. The results present an almost complete disagreement from those views toward which the preceding sections have led us. They are not concordant among themselves, except in the smallness of the circulation rates and stroke volumes which they indicate. They are so far from grouping themselves under any definable principle, that one might infer from them that the heart may normally make nearly any size of stroke, large or small, at any rate of beat.

The size of the stroke volume was one of the problems which interested Ludwig, and evidence bearing on it appears in many of the classic papers of his pupils such as Pawlow, Dogiel, Bohr, Stolnikow and others. (See Tigerstedt's review, 1905.) Stolnikow in particular used a simplified circulation in which, after tying off all the arteries except the axillary, he caught and measured the blood flowing from this one vessel, and then returned it to a vein. The results obtained indicated a stroke volume of from 0.32 to 1.6 cc. per kilo body weight, with a general value less than 0.64 cc. Zuntz states that the work of Ludwig and his pupils indicates a capacity in the heart to vary its stroke tenfold, and actual variations of threefold under nearly uniform conditions.

Dogiel attempted to use a stromuhr in the aorta, but with commendable and rare frankness declared his results valueless on the point which is here of interest. R. Tigerstedt (1891) later employed the same method and also expressed doubt of the significance of the results, which showed values of 0.27 cc. per kilo body weight on the average of what he regarded as the best experiments, and 0.42 cc. for the average of all. As Tigerstedt (1905) points out, the smaller value would afford a blood flow of such insufficient amount as to drive one to the hypothesis, which Bohr and Henriques supported, but which is now no longer supported by any investigator (since Pütter, 1911), that the normal blood flow is not sufficient to supply to the tissues as much oxygen as the body actually consumes, and that therefore a considerable combustion must take place in the lungs.

In his review in the *Ergebnisse der Physiologie*, R. Tigerstedt (1905) quotes extensively the work on the circulation rate in the rabbit, as measured by a stromuhr inserted in the aorta, carried out by Carl Tigerstedt. This work was certainly of the highest quality as regards technical skill and care; and yet it would be difficult to find more widely varying measurements of any function than are there reported. The general impression, which this and similar work by many other investigators in this field makes upon one who turns to it for information, is that the rate of heart beat has so little relation to the circulation rate, and that the stroke volume therefore is so variable that any precise formulation of relations is excluded.

In considering the results obtained in this field by Ludwig's pupils and their successors, we must keep in mind that the animal under experiment is usually immobilized, morphinized, anesthetized, partly bled, cold, over-ventilated, and in general more or less advanced in that depression of vitality which (as we now know) is most significantly indicated by a decreased venous pressure. Decreased venous pressure indicates decreased venous return to the right heart, and induces in consequence a subnormal and varying diastolic filling and stroke volume of the heart.

Even under such abnormal conditions evidence of value may be obtained on points connected with arterial pressure, the vasomotor nervous control and the relation of the heart action to arterial pressure. But in their bearing on the circulation rate and the stroke volume of a normal man or animal in healthy rest or muscular activity, the great mass of observations in the literature of this branch of our subject is essentially misleading, for the observations insidiously substitute the data of depression, subnormal venous pressure and the first stage of shock for the data of health. Arterial pressure affords no reliable indication as to whether the circulation rate is of normal or somewhat subnormal value; only when the venous return and circulation rate fall beyond a certain point is

arterial pressure necessarily affected. Furthermore, in many of these investigations the distinction has not been sufficiently drawn between increased circulation rate effected by certain procedures, such as compression of the abdomen, or peripheral nerve stimulation, or infusion of adrenalin, as a momentary effect and as a continuous factor; for these procedures will raise venous pressure momentarily, but if continued do not maintain it; while only transfusion and hypercapnia appear to be able to achieve this effect in a manner at all imitating the high venous pressure (10 to 20 cm. of water) of muscular activity in normal life.

There is, however, a criterion for judging when there is and when there is not an adequate venous supply. Lying on his back a normal man exhibits a pulsation at the base of the neck which is almost wholly venous. The jugulars are distended. But when he sits up, or stands, the neck veins are usually collapsed and do not pulsate above the upper line of the clavicle: normal venous pressure does not rise so high (v. Recklinghausen). Similarly in an animal at the beginning of an experiment the jugulars when first exposed are usually seen to be full and pulsating; but as a surgical operation on man or an experiment on an animal progresses, the fulness and pressure in the veins decrease and leave then collapsed.

The filling of the right ventricle, and thus in consequence the stroke volume of the left ventricle, decrease correspondingly and progressively. It is not, however, the absolute venous pressure which is here concerned, but the "effective venous pressure" which is the difference, as Henderson and Barringer showed, between intrapleural pressure and the pressure in the great veins at the heart level. It amounts in dogs to 50 to 70 mm. water, but is probably somewhat more in man. This effective venous pressure is the force which distends the right heart in diastole, and subject to the diastolic receptive relaxation of the heart itself, determines the size of the stroke volume, and thus the fulness of the pulse, but not arterial pressure.

It is the general neglect of the venous return and pressure which explains why experiments on the blood flow in animals under operative conditions have afforded such extraordinarily, variable, discordant and abnormally low values. If less blood is coming to the heart per minute than the heart can pump even at a slow rate of beat, it is evident that the heart rate may be accelerated to any extent without changing the minute volume. On the other hand, if the heart rate remains the same, and some procedure is effected so that a venous return, which was at first very low and inadequate, is restored more nearly to an adequate volume, the circulation rate may vary widely even while the heart rate remains the same. This affords the explanation for the irregular observations on the circulation rate under operative and experimental conditions of which the literature is full. A normal venous pressure is a necessary condition for a normal circulation rate.

Probably 90 per cent of all the blood pressure and blood flow experiments in the literature of physiology were performed on animals in the first stage of shock; just as in nearly all patients who are under etherization and operation for more than one hour the superficial veins nearly or quite disappear, the skin is pallid (cutaneous capillary depletion) and arterial pressure tends downward.

The depressant action even of anesthesia without operation is shown by recent (unpublished) observations of Haggard in this laboratory; for in two dogs under morphine and ether in good condition prior to operation, the stroke volumes were found to be only 0.83 cc. and 0.78 cc. per kilo body weight. Analyses of the

ether content in the inspired and expired air and in the arterial and venous blood furnished the data for these calculations. Similarly Doi (with Barcroft) found the oxygen utilization to be 5.5 volumes per cent and the stroke volume of the heart only 0.74 cc. per kilo in a cat under urethane (see also p. 180).

**OBSERVATIONS ON THE HEART-LUNG PREPARATION.** The heart-lung preparation, or reduced circulation, was first utilized by Newell Martin and was adapted for investigation of our problem by his pupils, Howell and Donaldson. A cannula is tied into the aorta close to the semilunar valve, and is connected to a rubber tube leading to an elevated reservoir, in which the blood may be collected and measured. A similar arrangement, connected to a cannula tied into the superior vena cava, supplies defibrinated blood to the right heart under any desired head of pressure. All other systemic arteries and veins are tied off so that the blood circulates only through the heart and lungs, which are ventilated artificially, and through the rubber tubes and glass vessels. Howell and Donaldson found, as have all other investigators, that the stroke volume is scarcely at all affected by variations of arterial pressure; the heart discharges as large a volume against a high as against a low pressure. On the other hand, the stroke volume was found to be absolutely dependent upon the pressure at which the blood was supplied to the right heart. Howell and Donaldson estimated the stroke volume of the heart for dogs at 1.17 cc. per kilo body weight at 180 heart beats per minute; but in their experiments this volume was not reached in most cases until abnormally high venous pressures were applied. At normal venous pressure (5 to 7 cm. of water) the stroke volumes were only 0.5 cc. per kilo or less. This relation of venous pressure and stroke volume may be illustrated from one of their experiments, in which the heart of a dog of 8.6 kilos at rates of beat of  $91 \pm 4$  per minute behaved as follows (with the stroke volume in cc. per kilo calculated by the reviewer):

Venous pressure, cm.	10	20	30	40	50	60	65	70	10
Stroke volume, cc.	2.51	5.08	6.42	8.39	9.4	10.97	10.8	10.8	2.38
Stroke volume, cc. per kilo	0.29	0.59	0.74	0.97	1.09	1.27	1.25	1.25	0.27

The largest stroke volume, at 60 cm., was thus only 1.27 cc. per kilo, while that at 10 cm. a figure already above the normal effective venous pressure was less than 0.3 cc. per kilo. Edema of the lungs (due we may guess to the high venous pressure found necessary to overcome the abnormal undistensibility of the heart) brought many of the experiments to a speedy end.

The importance of the phenomena of muscular relaxation, particularly in relation to the total amount of work possible in a given time, has only recently begun to receive recognition. It is *a priori* probable that the speed of a runner over any short distance is dependent upon the velocity with which his muscles relax, and allow themselves to be stretched, quite as much as upon the velocity of their contraction. In similar fashion in the heart, the velocity and extent of relaxation, in other words, the ease with which the muscle stretches under the distending force of venous pressure, is probably quite as important a factor in the heart's behavior as the force and rapidity of the systolic contraction. Cannon (1911) has emphasized somewhat the same behavior in the stomach which he terms its "receptive relaxation." In the heart, as in the stomach, the diastolic receptive relaxation is a vital factor and not merely a mechanical stretching like that of a rubber bag. Being vital, it is variable. In the experiment above quoted the data of venous pressure and stroke volume show the heart to have been so abnormally resistant to distention that we must regard its behavior as outside the field of normal physiology and as belonging to an artificial pathology. As the blood employed in the experiments of Howell and Donaldson was necessarily excessively aerated, and its content of  $\text{CO}_2$  and the ratio of  $\text{H}_2\text{CO}_3$ :  $\text{NaHCO}_3$  were thus abnormally reduced, the behavior of the heart is explicable as the now well demonstrated result of such alteration in the blood.

Evidence obtained by a similar technique by Starling and his pupils in a large number of papers has led Starling to formulate what he terms the "law of the heart." It is an extension of the all or nothing law and defines the conditions determining the force of contraction; it were better called, therefore, the law of systole. In its strictest formulation Starling in his Linares lecture defines it thus: Within physiological limits the larger the stroke volume of the heart, the greater are the energy of its contractions and the amount of chemical change at each contraction. In other words, the energy of contraction however measured is dependent upon the length of the muscle fibers. Any increase in the extent of active surface increases the energy of change, exactly as in striated muscle. Thus the uniformity of stroke volume against varying arterial pressures, which all investigators have found, depends upon the fact that, while the first few heart beats after a rise of pressure are slightly subnormal in amount, the consequent increase of the volume of blood in the heart calls into play greater power of contraction. In a similar way this law explains the capacity of the heart to accept widely varying amounts of blood from the venous system, and to increase the amplitude of its strokes so as to pump the whole increased volume from the venous side over into the arteries. In the main the formulation, as Starling states it, is merely qualitative and not developed in terms of cubic centimeters per kilo nor in millimeters of venous pressure. His conception is that, practically speaking,

the stroke volume is determined by the venous return and pressure, and that it is a widely varying function even in the normal individual under uniform conditions.

The behavior of the heart in the heart-lung preparation appears on the whole to be quite different from that which the preceding pages have given us good reason to believe prevails in the normal living man. While this technique throws much light on the inherent capacities of the heart, it affords also an illustration of the errors resulting from neglect of the doctrine established by Pawlow in his *Work of the Digestive Glands*, that the behavior of an organ can only be inferred with certainty from observations made while it is functioning in the body of a living, healthy, happy, unanesthetized animal. As Haldane (1922) expresses it, "The various activities of a living organism can not be inferred in isolation from one another, since in the living body organic regulation dominates them." Thus "preparations from the bodies of animals" and experiments on "fragments of animals" are often essentially misleading.

Starling gives a definition of tonus which seems to the reviewer to be merely a rhetorical circle. It is the "fitness of the muscle fibre," and its measure is the "energy set free at each contraction." He considers it as a condition largely independent of the extent of relaxation and contraction and correspondingly lacking any necessary relation to the size of the heart's chambers at the time. This view is quite different from that which the volume curve reveals, and different therefore from the usual meaning of tonus in respect to striated muscle and to such hollow organs, analogous to the heart, as the stomach, bladder and blood vessels. Tonus in general is best defined as the height of the coefficient of elasticity; and hence the degree of resistance to deformation.

The conditions in experiments on the heart lung preparation are abnormal in the entire elimination of the cardiac reflex nervous control, and in the replacement of the influence of the arteriomotor and venopressor mechanisms, the arterial resistance and pressure and the venous return and pressure, by arrangements of glass and rubber. They are also abnormal in that, while the blood is aerated in the lungs and thus adequately oxygenated, it is over-ventilated as regards  $\text{CO}_2$ ; for the only considerable source of  $\text{CO}_2$  in the experimental arrangement is the heart itself. Thus not only is the  $\text{CO}_2$  content of the blood reduced very low, but the  $\text{CO}_2$  ratio ( $\text{H}_2\text{CO}_3 : \text{NaHCO}_3$ ) and the H-ion concentration are abnormally reduced. The heart is thus rendered abnormally resistant to distention and abnormally high venous pressures are usually necessary. (Henderson, 1908; Jerusalem and Starling, Kaya and Starling, Moore, Patterson, Mansfeld, Mathison, Itami, Hooker, 1912.) On the other hand, the arrangement has distinct advantages in the ease of adjustment of arterial resistance and venous supply.

When we turn to the detailed data of Starling's experiments, and compare the venous pressures employed and the stroke volumes obtained with those of a normal man or animal, we find that the blood flow in cubic centimeters per kilo body weight (figures which unfortunately are not calculated in the originals) are not only very variable in different experiments and papers, but also are often so abnormal that they would be entirely incompatible with normal life. Thus in many experiments the stroke volumes figure out to less than 0.5, or 0.4, or even 0.3 cc. per kilo body weight at the normal effective venous pressure of 5 or 6 cm. water. They are indeed larger, but often less than 1 cc. per kilo, at the relatively high pressures of 8 to 10 cm.; and in many experiments they do not amount to as much as 1.5 cc. per kilo body weight until the enormous and abnormal venous pressures of 20 or 30 cm. are reached; and then the heart breaks down or, as Starling puts it, is fatigued, although the stroke volume and the work done by the heart are not greater than it maintains for years during normal life. On cats a maximal stroke volume of 1 cc. is found. The body weights are not given, but if we assume 3 kilos, the stroke volume and circulation rate would figure out to quantities which would be even smaller (in cubic centimeter per kilo body weight) than in the dogs' hearts studied.

We have here then the same phenomenon as that exhibited in the experiment of Howell and Donaldson above quoted. The essential points against all such data are two: 1, The normal effective venous pressure in a small animal during rest is only 5 to 7 cm. water. 2, If the stroke volumes at such pressures during life were less than 0.3 cc. per kilo body weight, the coefficient of oxygen utilization would rise to 100 per cent, and the man or animal would die of asphyxia; unless, as Tigerstedt has pointed out, Bohr was correct in claiming that there is normally a large pulmonary oxidation.

In fact, however, it is exceedingly probable that such observations are simply errors due to faulty experimental technique, and that the hearts were abnormally resistant to distention owing to their perfusion with blood in which the  $\text{CO}_2$  content was abnormally low. That this was the case is clearly proved by the experiments of Lovatt Evans (with Starling) in which the blood gas analyses show that the  $\text{CO}_2$  content of the blood was reduced to one-third or even a quarter of the normal. Henderson (1908) showed, and Jerusalem and Starling confirmed the observation, that extremely acapnial blood has this effect upon the heart, although in later work Starling and his collaborators have sometimes neglected this condition.

In investigations which Henderson and Prince (1913) carried out upon the excised cat's heart the same small stroke volumes, 1 cc. or less, were observed at first in hearts perfused with diluted sheep's blood; but when this blood was not only oxygenated but also impregnated with at least 50 volumes per cent of  $\text{CO}_2$ , the stroke volumes were increased to 3 or 4 cc. for each ventricle at venous pressures of only 5 or 6 cm. of blood; and the hearts continued to beat in this way for hours. It was found that even a small amount of ether was deleterious, and in the best experiments sudden decapitation without the slightest previous anxiety, pain or anesthesia, was employed before excision of the heart.

In their investigations on cats' hearts perfused with oxygenated and adequately carbonated sheep's blood, Henderson and Prince found that the left ventricle behaves in a manner in general accord with Starling's

law, but at a very much lower range of pulmonary venous pressures than those which one infers (if the left ventricle behaved at all like the right) from the papers of Starling and his collaborators. In the right heart, on the contrary, the maximum stroke volume (three times larger than any obtained by Starling and his collaborators even at abnormally great venous pressures and without the "fatigue of the heart" which they met) was attained at about 5 cm. pressure. Above this pressure all beats were maximal. Below this level the stroke volume varied with the pressure. The left heart was capable of taking more than the right heart was capable of sending. The general conception reached was that, while the left ventricle is the chief motor, the right ventricle is essentially the meter of the circulation. How this meter is operated and controlled is best shown in studies of the volume curve of the ventricles.

**THE VOLUME CURVE OF THE VENTRICLES.** Douglas and Haldane in the paper previously quoted have furnished strong evidence that the initial description of the volume curve (Henderson, 1906) affords an essentially correct picture of the behavior of the heart in a normal man. The general features of the circulation rate shown by this method differed, not so much qualitatively as quantitatively, from those found in most of the investigations upon animals quoted in the preceding pages. This result was due to the observance of certain precautions, without which the results of this method would also show the same lack of simple relation of heart rate and stroke volume to circulation rate, the same necessity for abnormally great venous pressures, and the same extension of Starling's law far above normal limits, which the investigators quoted in the two preceding sections have generally found under operative conditions. The precautions were prevention of acapnia, and assurance of a normal venous supply and pressure, as indicated by the upstroke of the volume curve, expressing the diastolic receptive relaxation and filling of the heart. A number of other investigators (Wiggers, Straub, deHeer, Socin, Schram) have studied the volume curve and have utilized it for important contributions to analysis of the heart's mechanics, normal and abnormal. Most of this work lies beyond the immediate scope of this review. The chief points to be here considered are concerned with the diastolic filling of the heart as the underlying condition determining the stroke volume (Henderson, 1908, 1909; Henderson and Barringer).

Harvey's sole considerable mistake (so far as we yet know) in describing the heart beat lay in the statement that it is the contraction of the

auricles which fills the ventricles. The volume curve, on the contrary, demonstrates that the greater part of the filling takes place during the earlier part of diastole, the period immediately after the systole of the ventricles; the next following systole of the auricles usually adding very little blood to the ventricles, although having other important functions not here in question (Henderson and Johnson). Venous pressure, not the force of auricular systole, is the agency which distends the ventricles.

If the heart is capable of varying its amplitude of stroke tenfold, or even threefold, as has been held by Ludwig, Zuntz, Starling and others, it is essential to learn the controlling conditions. In the respiratory mechanism, in which very large variations do occur, the variations of tidal volume depend upon the innervation of the diaphragm by the phrenics and upon the intercostal muscles and nerves. Do the cardiac nerves exert a similar control over the amplitude of heart beat? Henderson and Barringer utilized the volume curve technique to show that the cardiac nerves have no such power over the heart, and that it is only through variations of rate of beat that the vagi and sympathetics influence the amplitude of beat and the stroke volume. When the rate is so rapid as to leave insufficient time for full strokes, stimulation of the vagi by slowing of the rate of beat and lengthening diastole, may allow larger strokes. At very rapid rates further acceleration induced by stimulation of sympathetic fibers, by abbreviating diastole, may leave inadequate time for relaxation and filling, and may thus decrease the amplitude of beat. Aside from such effects, however, the cardiac nerves have little direct influence upon the stroke volume, although Wiggers and Katz (1920) and Wiggers (1920) have found that the duration of the periods of the cardiac cycle may be influenced.

The relation, which the volume curve shows between venous pressure and stroke volume, is that the rapidity and extent of diastolic filling increases progressively from zero pressure up to an effective venous pressure about equal to that existing in a normal man or animal, that is up to about 5 cm. of water in the dog. At this critical value the stroke volume in dogs is about 1.5 to 2.0 cc. per kilo body weight, and makes practically no increase with further increase of venous pressure within physiological limits; during healthy life all beats are therefore of about maximal amplitude. Below the critical level lies the first stage of circulatory depression; in this state occur all the bewildering and at first sight lawless relations which have usually been found between stroke volume, rate of beat and circulatory rate. It is probable that

the circulation in a person who is ill behaves thus. The second stage of still less adequate venous supply and filling of the heart, and consequent fall of arterial pressure, is the essential circulatory feature of well-defined shock.

In general Wiggers and his collaborators have confirmed this conception of the dependence of the filling of the ventricles and their stroke volume upon venous pressure. Wigger's observations on hemorrhage (1910) are especially valuable analyses of these relations. Wiggers (1914) and Wiggers and Katz (1922) have attempted, however, to show that the critical venous pressure, that below which the stroke volume is not maximal but variable, is much above the normal effective venous pressure; and this would mean that the stroke volume in normal life is a widely varying quantity. Examination of their protocols and records shows that these observations were made on hearts which, like those in the experiments of Howell and Donaldson, Starling and others, were abnormally resistant to distention. At normal venous pressures the circulation rates were too small to provide the amount of oxygen which the body requires to support life—unless Wiggers is prepared to adopt the Bohr theory of a large consumption of oxygen in the lungs. Like a great part of all the evidence in the literature regarding blood flow, his experiments on this topic are misleading because made when the subjects were moribund.

The volume curve also demonstrates that the rate of relaxation of the heart in diastole, as expressed in the up-stroke of the curve, is quite as important a feature of the heart's behavior and effectiveness as a pump, as is the systolic contraction expressed in the down-stroke of the curve. It is essentially similar to the relaxation curve of a striated muscle in an isotonic contraction curve: very steep at first, a bend, and then a part approaching a horizontal line. Diastole is thus found to consist of a period of rapid relaxation and filling, and a period of diastasis (Henderson, 1906) in which the dilatation is much slower or even, when this period is prolonged, comes to a standstill. Diastasis may therefore be abbreviated by increase of heart rate without considerably diminishing amplitude of stroke. The extent of the systolic contraction also increases, and the volumes of blood in the heart at the end both of systole and of diastole decrease, with increasing rapidity of beat and the accompanying increase of tonus. Variations of the duration of diastasis afford the principal explanation of the fact that the normal human heart in such a subject as Douglas (see table 1, B) is capable of the same volume of stroke at 60 and at 150 beats per minute.

Rates of beat so rapid as to allow no time for diastasis, and even to cut into the period of rapid relaxation, necessarily involve an abbreviated amplitude of diastolic relaxation and a corresponding decrease of filling and of stroke volume. At such excessive rates of beat, although the product of stroke volume and the rate of beat, that is, the circulation rate, may for a time increase still further, a decrease must ultimately occur as the heart approaches a condition bordering on tetanus. The less rapid the diastolic relaxation of the individual heart, the sooner this condition must develop. If an old man's heart relaxes slowly, his capacity for physical exertion is thus limited (compare p. 189); for the circulation rate would not be accelerated proportionally to the pulse rate during exertion, even though the systolic contractions were still like those of youth.

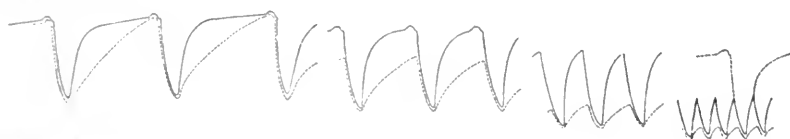
There are distinct differences between the cardiac volume curves of the cat and dog, the two animals which have been studied and from which we infer the behavior of the human heart; in the cat diastasis is generally a comparatively slight feature at normal heart rates, while in the dog's heart it is usually a definite period. From these facts it may be inferred, in accord with the capacities of the two animals for exertion, that a dog's heart with increasing rate of beat merely abbreviates diastasis without decreasing amplitude of stroke, and thus increases the circulation rate: hence the dog's capacity for sustained exertion. In the cat, an animal capable of sudden and intense but only brief exertion, an increase of heart rate necessarily decreases amplitude of stroke, and can increase the circulation rate comparatively little.

The human heart certainly behaves in a manner equally characteristic for the species man; but, as its volume curves can not be directly recorded, we have to infer the shape of the curve, and hence the capabilities of the heart, indirectly. From the durations of systole and diastole at various rates of beat as measured in pulse curves, the writer has found (unpublished observations) that in some persons the shape of the volume curve may be plotted; indicating conformity to a uniform or "superimposable" type curve. Evidently the heart of a vigorous man, which has a duration of systole of 0.2 to 0.25 second and is capable of beating 150 times a minute, or 0.4 second per beat, without considerable abbreviation of the duration of systole or lessening of amplitude of stroke volume, is an organ of very rapid diastolic relaxation and filling. The capacity of an athlete for intense and prolonged exertion must be founded in part upon the rapidity and extent of the up- and

down-strokes of the volume curve of his heart, and upon a long diastasis during slow pulse rates: a volume curve during rest shaped almost like a succession of mathematical root signs.

The claim that the volume curve can not be a true index of the stroke volume (Gesell) is refuted by the fact, established by Rothberger, that the circulation rate calculated from a volume curve, and that from a stromuhr inserted in the aorta, with a reasonable allowance for the coronary blood flow, give nearly identical measurements of the circulation rate. (For the special type of tambour required to record the volume curve without appreciable error, see Henderson and Barringer.)

PERICARDIAL VOLUME



COMPLETE CONTRACTION

Fig. 1. Typical volume curves at slow, medium and rapid heart rates, with the corresponding changes of tonus, and of systolic and diastolic volume. The lower dotted curves illustrate the effect of a slow diastolic filling, either from slow relaxation, or low venous pressure. The upper dotted curve at the right illustrates the conception of a uniform, superimposable, normal volume curve. If the first three beats at the left indicate a circulation rate of (say) 1.0 liter per minute, the next would give 1.6, the next 2.5, and the rapid beats at the right 3.0 liters per minute. The corresponding dotted curves with slow diastolic relaxation and filling indicate circulation rates of 1.0, 1.0, 0.9 and 0.8 liter per minute. To see the similarity of volume curves to isotonic muscular contractions, look at them upside down.

The volume curve has the same form as an isotonic muscle curve, as Frank first showed. This indicates that in the contraction and refilling of the heart, the volume of blood in the ventricles varies directly as the length of the cardiac muscle fibers. On geometrical grounds it has generally been held that the volume must vary as the cube of the length of the muscle fibers. This would of course be necessarily true, if all diameters varied proportionally; but such is not the case, and the heart would be a far less efficient pump if it were: in fact, the dorso ventral diameter may even increase during systole. If all diameters varied proportionally, the shape of the volume curve would be quite different from the isotonic form.

In some of the best records of the volume curve it was found (Henderson, 1908) that at various rates and amplitudes of beat the volume curves were longer or shorter arcs of a nearly uniform type curve

obtained during slow and full, or vagal beats. Wiggers and his collaborators have subjected this theoretical conception to a searching experimental critique, and have shown that under experimental conditions it does not hold at all precisely. Nevertheless in his recent discussions of the mechanics of the heart, Wiggers seems to keep this conception as a background, and to regard it as the underlying principle of normal cardiac behavior, as the reviewer believes it to be. It is probable, however, that in spite of every precaution in maintaining vitality in an experimental animal, even the best volume curves obtainable fall short of the normal steepness and extent of the diastolic up-stroke; for a dog must have the capacity, as a man certainly has, but as only the most spike-like (vagal) strokes in the volume curve would allow, to increase the rate of beat to 2.5 or even 3 times the resting rate without decrease of amplitude of stroke.

Some years ago Prince and Henderson devised a cardiometer with a soft rubber bag inside it fastened at the edge of the window. The apparatus could be placed over the heart and the thorax again sewed up without leaving a pneumothorax. The air tube connected with the space between the bag and the cardiometer was led out through the body wall without leak. The work was not published because, after the animals had come out of anesthesia with the cardiometer inside of them, they were not vigorous enough to do more than to stand or walk slowly, instead of running on a tread mill as intended. As they were unfit for vigorous exertion after so large an operation, it was soon necessary to anesthetize and kill them. The volume curves of the ventricles recorded from them, however, were in all essential features identical with those previously published.

**THE VENOPRESSOR MECHANISM.** One of the principal contributions which recent years have brought to our topic has been the general recognition of the importance of the volume and pressure of the venous blood stream returning to the right heart (Henderson, 1908, 1910; Mann; Dale and Laidlaw, Dale). The conditions, whatever they are, underlying the venous return may be conveniently termed the venopressor mechanism. It is the volume and pressure of the venous return which, combined with the diastolic receptive relaxation and distensibility of the right ventricle, determines the stroke volume. It is this factor in the circulation rather than any variation in the heart itself, which determines what clinicians, after feeling the pulse, term "the force of the heart beat." What is the nature of this mechanism?

Ludwig and his pupils, and following them all physiologists down to recent years, assumed without the slightest suspicion of a possible error or omission that regulation of the circulation falls almost wholly under the control of only two mechanisms: the cardiac and the vasomotor. If the heart was beating vigorously and "blood pressure" (meaning arterial pressure, the use of a false term illustrating the error we are discussing) was at a normal level, then the circulation was assumed to be normal. In fact, however, the venous return to the heart is, as first shown in connection with studies on the volume curve (Henderson, 1908 et seq.), a factor in the circulation largely independent of arterial pressure and under a control distinct from that of the vasomotor (arteriomotor) nervous system.

This mechanism is as yet incompletely analyzed, but seems to be located in the capillaries and venules, and is apparently, like respiration, largely under chemical control. Like respiration also, it is very delicately adjusted and is easily depressed by anesthesia (Henderson, Haggard and Coburn), over-ventilation (Henderson, 1908 et seq.), cold (deAlmeida), circulatory obstruction (Janeway and Jackson, Erlanger and Gasser), hemorrhage (Henderson and Haggard, 1922), histamine (Dale and Laidlaw), the products of tissue destruction (Cannon, 1922), and other even slightly depressive conditions. It is extremely probable from such investigations as those of Romberg and Pässler and Macallum (for literature see Hewlett, 1919) that in the circulatory depressions of acute disease it is the venopressor mechanism which is usually involved, and neither a cardiac depression nor a vasomotor depression like syncope.

The vasomotor (arteriomotor) mechanism is in fact usually extremely resistant to depression, as has been conclusively demonstrated by W. T. Porter.

In accord with this are the facts demonstrated in this laboratory that the well-recognized procedures for increase, and maintained increase, of activity of the vasomotor mechanism, such as direct and reflex nerve stimulations, adrenalin, nervous excitement, etc., have only a momentary effect, but no considerable continuing effect on venous pressure; while the conditions above mentioned as depressing the venous pressure and return are compatible even with excessive vasomotor activity.

Owing to the valves in many of the systemic veins, bodily movements exert a pumping action and propel the venous blood toward the right heart. The inspiratory movements of the thorax and the contraction of the abdominal muscles in deep breathing have a similar

effect. A general vasoconstriction by increased activity of the adrenals—if, as Cannon (1911) holds, this occurs during fear or anger—may temporarily increase the venous flow and pressure. A splanchnic constriction under the increased innervation of excitement or exertion may act similarly. But all of these factors together appear to be quite insufficient to produce the gradually rising and sustained venous pressure, and the augmented flow to the right heart which, coincident with an accelerated pulse, produce the increased rate of circulation during muscular work and augmented respiratory metabolism.

The most hopeful field in which to look for the now unknown essential factors in the venopressor mechanism, apart from the vasomotor nervous control of arteries and arterioles, is that of the rapidly accumulating knowledge of the independent contractility of capillaries and of such circulatory effects as those of histamine (Dale and Laidlaw, 1919). Thus Dale in his Harvey Society lecture (1919), in speaking of histamine shock, shows that the failure of the circulation is due to relaxation of capillaries, stagnation, decreased venous return, inadequate diastolic filling of the heart, and consequent failure of systolic output. As Krogh also (1919, 1922) truly says in speaking of shock as due to capillary dilatation: "Nothing can be more striking than the contrast between . . . arteriole dilatation and capillary dilatation respectively." These statements are essentially similar to those of the reviewer (Henderson, 1908, 1910).

Many of the investigators quoted in this review have recorded their opinion that the circulation rate is correlated with the degree of muscular activity, oxygen consumption and  $\text{CO}_2$  production. In general there is a tendency to recognize that  $\text{CO}_2$  is a more potent influence (both through its influence upon H-ion concentration, and through specific qualities) than is oxygen in the regulation of the circulation, as Haldane and others have shown it to be in the adjustment of respiration. The evidence will therefore be here briefly cited which indicates that the amount of  $\text{CO}_2$  in the tissues and venous blood is the controlling factor in the regulation of the venopressor mechanism (whatever its nature) during the activities of normal life.

The writer has had peculiarly favorable opportunities to make observations on the rise of venous pressure coincident with increase of  $\text{CO}_2$  in the body in normal men, while testing so-called oxygen helmets for the U. S. Bureau of Mines. If the alkali cartridge is defective, the exhaled  $\text{CO}_2$  accumulates in the apparatus and in the man's body while he breathes an atmosphere rich also in oxygen. A marked rise of

venous pressure and dilatation of veins always result. The same extent of venous dilatation, i.e., relaxation of the veins themselves, occurs in a Turkish bath; but under mere heat the rise of venous pressure is absent. Studying men in the laboratory, Henderson, Prince and Haggard found that the measurement of the venous return in man is most effectively and significantly made in the head down position. When thus observed after forced breathing the venous column was lowered 8 to 11 cm., an amount corresponding to the depression found by the same method in patients after anesthesia and operation. On the other hand venous pressure was markedly elevated alike after exercise and after inhalation of 6 or 7 per cent  $\text{CO}_2$ . The effects passed off slowly.

Schneider and Truesdell have found on eleven human subjects that when the alveolar  $\text{CO}_2$  was kept at 7 per cent (normal 5.0 to 5.7) and ample oxygen supplied, the venous pressure was increased 74 per cent. Capillary and arterial pressures also rose, while the volume of the hand and the blood flow through the hand decreased. When normal air was again breathed, venous pressure returned to normal much more slowly than arterial and capillary pressures. The authors say that "the rise of capillary and venous pressure . . . suggests an increased return of blood to the heart, and therefore a well-defined increase in the minute volume of blood flow from the heart. Contrary to this indication we find the heart output per beat . . . never clearly increased." Liljestrand also has found that in normal subjects inhalation of  $\text{CO}_2$  does not augment the circulation rate.

The fact that venous pressure may be distinctly increased in normal subjects, without increase of stroke volume, seems to accord with the view that the normal venous pressure is at or above the critical value, and is therefore sufficient to produce maximal beats. On the other hand, in patients depressed by anesthesia and operation, Henderson, Haggard and Coburn have obtained marked and rapid increase of the circulation, particularly of the venous return with apparently also a greatly increased stroke volume and striking changes in the skin capillaries under  $\text{CO}_2$  inhalation.

Henderson and Harvey attempted to analyze the problem of the nature of the venopressor mechanism, but the argument is too complex to repeat here. It was in part hypothetical and does not appear to have been convincing to others. In their experiments abolition of vasomotor control was obtained by section of the spinal cord in curarized dogs and was evidenced by enormous lowering of arterial pressure,

with but slight or no change of venous pressure. Electrical stimulation of the cord restored and maintained arterial pressure, but affected venous pressure only slightly. In a curarized dog stimulation of the central end of the sciatic induced a great rise of arterial pressure, but only a slight and temporary effect on venous pressure. In decapitated cats a slow injection of adrenalin raised and maintained arterial pressure but venous pressure was affected only temporarily and returned to a low level even during continuation of the injection. On the other hand, in decapitated cats maintained by insufflation of oxygen, the addition of 15 per cent  $\text{CO}_2$  produced only slight effects upon arterial pressure or the heart, but induced and maintained an enormously high venous pressure. There was no indication that the heart was injuriously affected, but rather the contrary. This experiment, in the reviewer's opinion, shows that the mode of coördination between the volume of the venous return and the respiratory metabolism is essentially through the peripheral effects of  $\text{CO}_2$  or, less probably, (as arterial pressure was not influenced) through spinal centers. But the problem is only in its beginning; for Krogh (1919, 1922) finds that the respiratory gases in physiological amounts have no distinct effects upon capillaries. His observations are, however as yet, chiefly upon the frog. Mall's observations are usually quoted as showing that the additional blood in muscles during work is drawn from the splanchnic area. But this explanation becomes less and less satisfactory (Henderson and Harvey), or even tenable (Burton-Opitz).

Mathison, Hooker (1918, 1920, 1922), Fuhner and Starling and others have worked on this and closely related topics. Their results are in general not very concordant with the conception of the nature of the venopressor mechanism above indicated (Henderson, Mann, Dale, Krogh) nor with each other, except on the broad fact which every one now admits that  $\text{CO}_2$  in excess and  $\text{CO}_2$  deficiency, whether acting directly or through the H-ions of the blood, have powerful influences on many factors in the circulation.

Dale and Evans have recently found that, in cats either decapitated or under urethane or paraldehyde, excessive ventilation induces a marked lowering of arterial pressure; but that this effect does not occur if the lungs are equally vigorously ventilated with air containing  $\text{CO}_2$  (human expired air). Venous pressure was, however, only slightly, if at all, affected; and they consider that the point of action is in the spinal vasomotor centers. They show that the effects are due to acapnia, and not to alkalosis.

In this connection attention may be called to the interesting experiments demonstrating the peculiar effectiveness of carbonic acid in contrast to other acids in its penetration of cells and influence upon protoplasm recently reported by Jacobs.

Whether the venopressor mechanism is some special mechanism or, as is still held by most investigators, merely a part of the general vasomotor mechanism, is a question for further investigation. Both experimental and clinical observations on man seem, however, to demonstrate that, both in the ordinary activities and work of human life and in the conditions of patients under anesthesia and operation,  $\text{CO}_2$  is the hormone somehow regulating the venous return.

OBSERVATIONS BY X-RAY. The x-ray shadow and photograph, and the orthodiagram have not afforded such complete and easily interpreted and measured data as might at first have been expected; and the limits of space here allow mention only of points directly concerned with our topic, with no attempt at a general survey of the large literature of x-ray observations on the heart.

It is a significant fact that no observer has reported, and we may be sure therefore that there do not exist, such variations in the amplitude of pulsation of the heart's shadow, between bodily rest and muscular exertion, as would be expected if the amplitude of the stroke volume is subject to a several fold variation. On the contrary so far as the evidence goes, it indicates on the whole a stroke volume of nearly uniform amplitude during rest and exercise.

Against the theory of much larger heart beats during exertion than during rest is also the fact that the shadow is generally slightly smaller (Dietlen), not only immediately after, but even during, muscular work (contrary to the initial observations of Nicolai and Zuntz, and Zuntz and Schumberg) than it is during rest. No larger beats should therefore be expected during work.

In the heart shadow, as Dietlen also says, "at the end of the systolic contraction the ventricles appear for an instant to stand still; . . . and at the end of diastole one sees (when the breath is held) that for a relatively long period the ventricles stand quite still." This first pause is a confirmation of the appearance of the volume curve (the blunt end of the spike) at the end of systole. The second and longer standstill is a direct demonstration of diastasis as a normal period at the end of diastole during slow pulse rates in man.

Even in the shadow of the human heart the maximum extent of movement of any boundary line is only 6 mm., and it is correspondingly

less in smaller animals. As the heart does not contract symmetrically, it is extremely difficult to transform such changes of area into values in cubic centimeters. In spite of this difficulty, Meek and Eyster have utilized the shadow of the dog's heart to estimate the variations in stroke volume after hemorrhage, and find practically no decrease in the shadow, but a distinct decrease by the volume curve, under a moderate blood loss. They have also applied the method to the behavior of the heart during an artificially produced plethora, and find an increase both of diastolic volume and of stroke volume in accord with Starling's law. Exercise, they find (1923), may increase the stroke volume by 20 per cent. But this amount is so slight that these observations really weigh strongly against the theory of a two or three hundred per cent variation of stroke volume. Increase of stroke volume coincident with decrease of diastolic size occurred in some cases: a fact incompatible with increased length of fiber as the cause of the increased stroke.

In like manner, Bruns, in a recent study of the heart's shadow of forty-eight human subjects during rest and muscular work, finds that in 75 per cent of his cases the heart is smaller after exercise than before exercise; and that during work it is larger in 15 per cent of his subjects, smaller in 25 and variable in the remainder. Even an increase of 10 to 40 mm. arterial pressure, and 10 to 50 beats per minute causes a maximal increase of shadow area of only 3.3 to 5.5 per cent. From these observations Bruns concludes that the human heart in the normal body does not behave as Starling and other investigators, working on animals under experimental conditions, have generally believed. He says that there is "a fundamental distinction between experimental and clinical observations" (meaning by "clinical observations" those made under the conditions of real life) regarding the behavior of the heart, for in order to produce 3 to 4 times as large stroke volumes during work as during rest, as these writers hold to occur, distinct increase of diastolic size would be necessary. That he would have found such an increase, if it had existed, Bruns shows by the fact that any decrease of venous return, as in Valsalva's experiment, is seen in a marked decrease of the area of the heart's shadow.

In regard to the critically important point thus demonstrated, it is noteworthy that Starling in his Linaere lecture (p. 25) admits that the increase of size (i.e., greater distention of the heart), which is necessary to call the "law of the heart," into operation and to produce larger strokes during muscular work, is "only temporary;" and he has

therefore to invoke increased arterial pressure and "a more abundant flow of blood through the vessels supplying the wall of the heart" to maintain the supposed increased extent of systolic contraction thereafter. This statement, however, to a large extent concedes the dissimilarity of behavior of the heart in the heart lung preparation and in the living body, and leaves little for the "law of the heart" to do in normal life.

#### PRINCIPAL CONCLUSIONS

Convincing evidence from a wide range of sources indicates that in vigorous men and other mammals of all sizes the stroke volume of the heart is a nearly constant quantity for each individual. Both during rest and work it is of the order of magnitude of 1.5 to 2 cc. per kilo body weight. Since this conclusion is, however, particularly as regards the stroke volume during rest, distinctly opposed to the opinion heretofore held (which assumed a variation of stroke volume of 200 to 400 per cent, or more), the following points of evidence upon which it chiefly rests deserve emphasis:

There is general agreement among investigators that during muscular work, and a high respiratory metabolism, stroke volumes of this order of magnitude are attained.

During rest the average oxygen utilization from the whole blood stream is, however, as we are now learning, much lower than was formerly believed, and the blood stream, or circulation rate, is correspondingly larger than had been inferred. This is strongly indicated by the newer evidence here reviewed. The oxygen utilization amounts to only 3 to 4 volumes per cent, or to a coefficient of 0.16 to 0.2 instead of 0.3 or more as generally held heretofore. With this fact established, there appears to be no other possible conclusion except that the stroke volume during rest is correspondingly larger than has been heretofore generally believed; and that it is of essentially the same volume during rest as during work. In other words, the stroke volume in a normal man or animal is practically a uniform quantity (within 20 or 30 per cent) during all the ordinary activities of life. The circulation rate is therefore nearly proportional to the pulse rate.

Reverting to views supported long ago by Hering and by Vierordt, but more recently neglected, it is here shown that in vigorous animals of various sizes the pulse rate and the circulation rate (measured in cubic centimeters per kilo body weight) vary, just as does the respiratory metabolism, in direct proportion to the surface : mass ratio; for the oxygen utilization and the stroke volume per kilo are nearly the same in mammals of all sizes.

The conditions determining the venous return, the so-called venopressor mechanism, are regulated by the CO<sub>2</sub> content of the tissues and venous blood. This mechanism and the regulation of the heart rate (the latter not here discussed) are the factors which adjust the circulation rate to the energy expenditure and respiratory metabolism during the various activities of normal life.

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## THE FUNCTION OF THE VESTIBULAR APPARATUS

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The vestibule, as distinguished from the cochlear or auditory portion of the internal ear, is a circumscribed peripheral receptor present in all vertebrates from the Ostracoderms of the middle Ordovician period on to the present. It includes the sacculus, lagena, utriculus, otoliths and semicircular canals with their ampullae. Its form changes somewhat with the type of animal chosen for study, the ampullar and canal portion being represented in the Cyclostome *Myxine* or *Bdellostoma* by one canal with two ampullae, and by two canals in *Petromyzon*. In the Ichthyosaurs and Plesiosaurs, fossil reptiles of the lower Mesozoic (Triassic and later), it is much larger than in any extant form, the dimensions of the canals being in centimeters rather than in millimeters or their fractions, as is the case in present day vertebrates. The general anatomy of the vestibule is given in the papers of Retzius (1), Ayers (2), Bigelow (3), Stensio (4) and others. Shambaugh's (5) plates give the vascular supply of the vestibule of the fetal pig in detail.

Herriek (6) has given four criteria of a receptor; namely, the anatomical, the physical, the physiological and the psychological. The case for the vestibule may be examined briefly with reference to the application of these four criteria.

Anatomically, as well as physiologically, the vestibule belongs to Sherrington's group of proprioceptors. It is protected from direct contact with any ordinary agencies, or from direct excitation by any ordinary changes, in the external environment. The agencies which constitute its biologically adequate and normal stimulus must ordinarily arise within the body itself. The general structure of the vestibule suggests that the fluid contained within its various cavities and the otoliths in its saccule and utricle may act upon its specific nerve endings through the medium of a mechanical pressure generated by the inertia of the fluid or otoliths when the head is moved in any of the various planes of space.

Regarded from the point of view of physics, the idea that mechanical pressure may be the biologically adequate stimulus is supported not

only by theoretical considerations (7), (8), (9) but by actual experiment as well.

Physiologically, excitation of the vestibule gives rise to certain objective reactions of the eyes, the muscles of the limbs and trunk, and of certain viscera. Not all of these reactions are peculiar to excitation of the vestibule, but some combinations of reactions can be found which do appear to be peculiar to excitation of the vestibule.

Considered psychologically, the view recently taken by psychologists is that there is no mental quality arising from excitation of the vestibule that is discoverable on introspection. The general mental picture arising from any of the various experimental methods of excitation of the vestibule is rather to be considered as the integration of the various organic results of vestibular excitation and the mental consequences of these organic results than as something due to the vestibular endings alone (10).

The literature on the vestibule is enormous, probably including in all its phases about two thousand separate papers. A complete bibliography alone would require more space than can be devoted to the entire paper. The most complete recent bibliography of the subject is given by Griffith (11). Only a part of the physiological reactions arising from excitation of the vestibule can be considered at this time, without going very far into the vexed question of its central connections in the nervous system.

Although the early work on the vestibule was done on birds and mammals and even on the human subject, it seems best to depart from the traditional historical method of presentation and consider the effects of vestibular excitation or extirpation with reference to its comparative relationships. Although no true phylogeny of function can be given until the paleontologist and systematist have given us a more complete and logical account of vertebrate ancestry and descent than is now available, there is still a sufficient amount of difference in the reactions to vestibular excitation at various levels in the animal phylum to make such a method of presentation worth while.

The vestibule has been generally considered to be related to the maintenance of equilibrium. It is evident, however, that it cannot be the main receptive organ for the maintenance of equilibrium in invertebrates, since they do not possess it. A general account of the mechanisms in invertebrates has been given by Bethe (12), who points out that some of them maintain their normal position in space merely by their structure, that is, the distribution of the mass of the body with

reference to its center of gravity. Some dead organisms will float in water in the same position as the living organisms, and their position will be reversed when put into a fluid of a different specific gravity. Other invertebrates have so-called auditory pits containing grains of sand or other solid matter. Kreidl (13) washed the sand out of the auditory pit of a crustacean and replaced it with iron filings. The animal's movements could then be controlled by holding a magnet near it in the water. A considerable number of invertebrates have well developed otocysts, removal of which entails severe disturbances in locomotion. The literature is given by Fröhlich (14), Prentiss (15) and others.

*Equilibrium in vertebrates.* Passing over the long and unfilled gap between vertebrates and invertebrates, and even over the lower forms of what some consider vertebrates, we may take *Amphioxus* as the next type.

*Amphioxus.* Willey (16), in describing this form, states that "Besides lacking differentiated lateral fins, *Amphioxus* differs fundamentally from the higher Vertebrates in the absence of a cranium, of paired eyes, and paired or unpaired auditory organs" (p. 17).

"*Amphioxus* exhibits the characteristic vertebrate bilateral compression of the body in a very typical manner. It is obvious that such a shape of body is highly unfavorable for the maintenance of the equilibrium except with the assistance of some special mechanical and sensory apparatus. Now in *Amphioxus*, the metapleural folds, whatever their exact function may be, do not serve in any way as balancing organs; and, as mentioned in the text, *Amphioxus* has no means of maintaining its equilibrium when not actually swimming" (p. 43-44).

It is perhaps because of the difficulty of maintaining a body of such form in equilibrium that the locomotion of *Amphioxus* is so irregular,—"a rapid, curiously irregular wriggle"—periods of swimming forward, sometimes upon the back, sometimes upon the abdomen in the position of ordinary fishes (17) being interrupted by somersault movements and backward swimming (18) until the animal settles down and burrows tail first into the sand (19).

*Cyclostomes.* Experimental removal of the vestibule of *Cyclostomes* has been done successfully but once. Ayers (2) removed the vestibule from *Bdellostoma dombeyi* and found no appreciable disturbances of locomotion. Some of the same considerations which apply to *Amphioxus* apply also to *Cyclostomes*. Although there is a somewhat rudimentary vestibule—whether primitive or degenerative does not make so much difference from our present point of view—the locomotion of

Cyclostomes does not partake of the character of the strong swimming fishes, and the whole motor mechanism—nervous and muscular, shows a less high stage of development. Like *Amphioxus*, *Bdellostoma* has no fins, and its body is more like the cylindrical *Balanoglossus* than like the bilaterally compressed *Amphioxus*. While it has the sense organs for the perception of the change of aspect of the head in space, it has no paired effectors or fins which may react in a special way to excitation of the vestibule. As Willey points out, the maintenance of equilibrium is easier when the body is cylindrical than when it is bilaterally compressed, as in *Amphioxus*. *Bdellostoma* swims with eel-like movements of the body, and when at rest on the bottom, curves some part of the body, usually the tail, to one side or the other to keep it from rolling over.

*Selachians.* The relatively large size of the vestibule, and the fact that the soft cartilage in which it is embedded makes operative approach easy, have been among the reasons why the Selachians or Elasmobranch fishes have been favorite subjects of experimentation. The methods of experiment have varied from complete removal of one or both vestibules, section of the eighth cranial nerve, removal of the canals and ampullae without injury to the utricle, removal of the utricle without injury to the ampullae, exposure and mechanical excitation of the otolith of the utricle, and testing the reactions of the fins and eyes to a change in position of the head when the vestibule is intact. Most of the observations have been made upon some genus of the dogfish and upon rays. All three canals and the utricle and the saccule are well developed in Selachians, and we have for the first time in the vertebrate phylum, a fully developed vestibule with all its parts as it exists in higher vertebrates. Paired fins are also present in Selachians, and we have again the first complete mechanism for the maintenance of the equilibrium of a bilaterally compressed vertebrate body—sensory mechanism, central nervous system and effectors which may act as balancing organs. The maintenance of the normal posture of fish requires some mechanism, as Manoyer (20) showed that fish swim in labile rather than stable equilibrium.

Such a balancing mechanism— a receptor, a central system and the effectors—fulfills Goltz's (21) conditions, and, although found for the first time in living vertebrates in the Selachians, exists in a modified but physiologically adequate form in such free-swimming invertebrates as the crayfish and the lobster. But it is well to state here that the vestibule does not constitute the only receptor which is concerned with

the maintenance of the normal position of any vertebrate in space. Other proprioceptors and the exteroceptors must be taken into account, and the maintenance of equilibrium is an act requiring a high degree of correlation of response (22) and a highly developed mechanism of integration in the central nervous system. It is only by keeping these things in mind that we may guard against either of two errors—attributing too great an importance to the vestibular mechanism, or denying its efficacy altogether.

The reactions of the eyes and fins of the dogfish with intact vestibule when rotated about any one of the three axes of the body—longitudinal transverse or dorsoventral—are too well known from the work of Lee (23), Maxwell (24), Lyon (25) and others to require more than a brief mention. It has been found that stimulation of the ampulla of one anterior canal causes the eyeball of the same side to roll upward and the other downwards, but the direction of rotation of the eyes about the anteroposterior axis is reversed. When the anterior canal is stimulated, the anterior pole of the eye of the same side rises more than the posterior; when the posterior canal is stimulated, the posterior pole of the same side rises more than the anterior. The direction of rotation of the opposite eye is always the reverse. Stimulation of the ampulla of the horizontal canal causes the eye of the same side to roll straight forward, and the other eye straight backward. Maxwell differs slightly from this account. According to him, the eyes of both sides rotate forward on their visual axes when the ampulla of a posterior canal is stimulated, and backward when the ampulla of an anterior canal is stimulated. According to Lee, the direction of rotation of the two eyes about their visual axes is opposite; according to Maxwell, it is the same when the ampulla of the anterior canal, for example, is stimulated.

Lee particularly, and possibly Maxwell, assumed that, in rotating a dogfish about any of the axes of the body, the ampullae and not the otoliths were stimulated. Breuer (26) had assumed that the canals and ampullae were concerned with the perception of movement in curved lines, i.e., angular acceleration, and that the otoliths were concerned in the perception of movement in straight lines, i.e., linear acceleration. This view was widely accepted, although no direct proof of its correctness had ever been given. In a later paper, Maxwell (27) described the reactions of a dogfish to rotation when the ampullae of all six canals had been removed without injury to the utricle. All

the compensatory movements of the eyes persisted after such removal of the ampullae, except those following rotation of the animal about its dorsoventral axis, i.e., in the plane of the horizontal canals. It seems clear, from Maxwell's results, that the otoliths are concerned, along with the ampullae, in the perception of angular acceleration.

The converse of this state of affairs,—reaction of the ampullae after complete removal of the utricular otoliths—was also tested out. When the utricular otolith is removed without injury to the ampullae, all the compensatory movements of the eyes in response to rotation of the animal about any one of the axes of the body persist.

After removal of the ampullae, with conservation of the otoliths, the compensatory movements of the eyes, although prompt, are noticeably slower than when the vestibule is intact. If seized while in the water, the animal strongly resists the attempt to turn it back downward; but one feels that this resistance is neither as prompt nor as strong as in the intact animal (27). In swimming there is a tendency to sway from side to side about the longitudinal axis like a boat insufficiently ballasted. When the otoliths alone are removed, with conservation of the ampullae, the compensatory movements are slightly slower than in the intact animal. Swimming is apt to be accompanied by a slight rocking movement from side to side; and if turned back downward in the water, the animal rights itself promptly, but may turn almost or completely over before coming to rest in the normal upright position.

The otolith of the utricle is more easily accessible in the shovel-nosed ray (*Rhinobatus*) than in the dogfish. It may be exposed and stimulated mechanically *in situ*, after removal of all the ampullae. Maxwell (28) found that "pressure on the right side of the otolith of either ear produces the same eye movement which results from rotation of the body to the left about its longitudinal axis," i.e., the right eye is depressed and the left rolled upward, "and that pressure on the anterior side of the otolith produces the same movement of the eyes which results from tilting the head upward,"—the eyes are rotated forward about their visual axes. It is the displacement of the otolith and not the weight due to the otolith which is the actual stimulus, and it is the direction of the displacement which determines the direction of the compensatory movement.

Maxwell's conclusions are that the assumption of a sharp differentiation of function between the otolith-bearing portions of the otic labyrinth and the semicircular canal portion is not justified by the facts.

The two parts of the labyrinth reinforce each other, for the reactions produced by either one alone are slower and less vigorous than when both sets of organs are intact. The two parts may not have identical functions, however, since response to rotation in a horizontal plane is completely lost after removal of the ampullae alone.

Removal of all ampullae and the otoliths of both utricles has the same effect as complete destruction of the vestibules or section of the eighth cranial nerves.

Some investigators have reported negative results from complete bilateral or unilateral destruction of the vestibule, but more recent work tends to show that there is a definite train of symptoms following such a lesion. All compensatory movements of the eyes and fins to rotation of the animal about any axis of its body are completely and permanently lost after bilateral lesions. The animal swims indifferently in the back upward or belly upward position. A weak animal comes to rest on the bottom of the tank on its side or back, but a vigorous animal will right itself on reaching the bottom, the reaction being due, in Maxwell's opinion, to the sense of touch. As he expresses it, the geotropic responses of the animal are lost, but the stereotropic responses are retained. Some degree of recovery may occur, but there is always some disturbance when the fish is made to swim rapidly, as a rule.

The effects of unilateral lesion are less severe. The eye of the injured side turns downward and the eye of the sound side turns upward  $20^{\circ}$  to  $40^{\circ}$ . The body is curved markedly to the injured side. In swimming, the fish may roll to the injured side. Normal swimming seems possible, but disturbances of locomotion, particularly when the eyes are closed, are very apt to appear. The displacement of the eyes is permanent as long as the animal lives—two weeks in some cases. Compensatory movements of the fins and eyes are still elicitable on rotation in any one of the three planes of space, although often weakened and difficult to observe.

Considerable discussion has arisen as to whether the effects of vestibular lesions are due solely to the loss of a peripheral receptor or due largely to the irritative effects of the injury. Gaglio (29), König (30) and Breuer (31), the last using pigeons, have applied cocaine to the vestibule. Gaglio observed, in the Mediterranean dogfish, the same results after the application of cocaine as after anatomical removal of the vestibule. This strongly indicates that irritative processes play but little part in the effect of vestibular lesions in this form. Again,

one would expect about as much irritation from destruction of the ampullae together with their specialized nerve endings as from complete destruction of the vestibule. Yet Maxwell found that the reactions of the animal, after removal of the ampullae alone, differed from those of the normal animal only slightly. Whatever effects of irritation may have been manifested elsewhere, there were extremely few which were apparent in those reactions which we have come to look upon as characteristic of vestibular excitation. It is my opinion that the effects of irritation in connection with vestibular lesions have been greatly over-emphasized. One might make the same statement with reference to many other lesions of receptors or parts of the central nervous system.

One other permanent effect of bilateral vestibular lesions was brought out by Gaglio, and that is the loss of muscular strength following this destruction. Parker (32) also remarks that the muscle tonus of the animal is noticeably reduced after bilateral lesions. This can scarcely be due to any effect on the muscles themselves, but rather to the loss of afferent impulses normally concerned in the maintenance of tonus or the utilization of muscular strength.

Both Parker and Maxwell agree that the saccular otolith has little or nothing to do with vestibular function proper. Parker regards it as a part of the mechanism of hearing in fish.

Although Mach showed the physical improbability, even impossibility of actual currents of fluid in the semicircular canals, the hypothesis of stimulation of the ampullar nerve endings by currents set up in the canals has been very popular among otologists. Maxwell freed the horizontal canal for nearly its whole length, ligated it near its posterior end and, after cutting it, raised it so that its plane was vertical. Rotation of the animal in a horizontal plane, about the axis of the canal in its new position, was attended by the usual reaction to rotation. No possible currents could have been set up in the canal under these conditions, and it is difficult to see how any pressure could originate in the canal to be communicated to the ampulla. In the statement of the mechanism of excitation of the ampullar endings, Mach, Breuer and Crum Brown supposed that pressure arising from the lag of the fluid in the canals during rotation was transmitted to the ampullae. Implicitly, it was assumed that the pressure arising in the utricle from the inertia of its contained fluid would either not be transmitted to the ampullae or, if so transmitted, would not be effective in stimulating the nerve endings. When one considers the mechanics

of the vestibule, there is no anatomical or physical reason discoverable for such an assumption. And, in view of Maxwell's results, there seems good reason for believing that the normal mechanism of excitation of the ampullar endings is the transmission of pressure from the utricle to the ampullae. In any event, it would seem time to give up, once for all, the idea of fluid currents in the canals under any ordinary conditions. On the other hand, we have Breuer's (31) statement that when rotation is sufficiently rapid or violent to set up actual currents in the vestibule, the ampullar endings would be injured, and the cristae acusticae swept from their places.

The evidence now available would seem to indicate that both ampullae and otoliths are capable of responding to angular and linear acceleration, and that the two mechanisms work together in this function. The otoliths, because of their greater specific gravity (Maxwell), would have a greater inertia than an equal volume of fluid, and there is no reason to suspect that their displacement might not occur in either angular or linear acceleration. There is no reason to suspect that the fluid in the utricle would not lag in either angular or linear acceleration, and thus give rise to pressure somewhere. It seems extremely probable that the transmission of this pressure to the ampullar endings constitutes their normal and biologically adequate mechanism of excitation.

*Teleosts.* Experimental work on the internal ear of teleost fishes has not been as extensive or carried out in as much detail as in Selachians, but the results at hand, so far as they go, are in keeping with the results obtained from the study of cartilaginous fishes.

Bigelow (3) showed by dissection that the ear of the gold fish, *Carassius auratus* L., is essentially like that of *Cyprinus*, as described by Retzius. There are certain anatomical differences in the ear of *Carassius* as compared with the ear of a shark, one of which is the presence of two otoliths in the combined lagena and saccule of *Carassius*. The wall of this sac, as Bigelow showed, is supplied by branches of the eighth cranial nerve. Many of the experiments on teleosts were done in the course of experiments on hearing, and the disturbances of equilibrium were described only incidentally.

Tullberg (33) considered that the ears of the bony fishes were not organs concerned in the maintenance of equilibrium, and that they had little to do with hearing. Their main function, in his opinion, is the perception of slight movements in the water, and particularly those arising from currents in the water. He bases his arguments on the

fact that bony fishes may, in time, recover almost completely from lesions of the internal ear. The immediate effects are therefore due to shock, and not to the loss of a particular receptor.

Parker (34) found that *Fundulus heteroclitus*, after section of both eighth nerves, lost the power of maintaining equilibrium when swimming rapidly, and swam in any position in spirals or even in circles. In his discussion of Tullberg's view, Parker (p. 201) admits that *Fundulus* may recover fairly well in a few days after the operation, so long as it swims slowly. But it invariably loses this power of maintaining equilibrium if made to swim rapidly in a large amount of water. The greatest length of life of any animal after operation was six weeks, but the effects were clearly observable throughout this period. Parker attributed the partial recovery of the animal and its facility in swimming slowly to increased use of the eyes. He admits that this is an assumption on his part, but there is much evidence drawn from the study of reactions in invertebrates to support such a view. Until this assumption is definitely shown to be untenable for bony fishes, Parker considers that Tullberg's conclusion is premature.

My own view is in accord with Parker's. The same arguments adduced against peripheral irritation and shock in the case of vestibular lesions in the cartilaginous fishes apply with equal force in the case of the bony fishes. It may be shown that the loss of any one sense does not entail any very severe permanent disturbances of locomotion in higher animals. One might conclude, therefore, that no one of these sensory mechanisms had much to do with locomotion. The argument should be carried a little further, however. If no one sensory mechanism has much to do with locomotion, it should be possible to eliminate all of them and still retain relatively unimpaired powers of locomotion. For if the whole is equal to the sum of all its parts, the whole effect should be relatively slight. Common experience points very strongly to the conclusion that the total effect is not relatively slight. Since Euclid's argument has now been subjected to criticism and analysis for some centuries without suffering serious damage, I am inclined to suspect that the fault may lie in some of our common assumptions concerning the functions of various sense organs rather than with Euclid's reasoning. The presumption is strong that some of the quantities concerned in physiological reasoning, e.g., the effects of removal of various sense organs, are really variables instead of constants, as Tullberg's line of reasoning assumes. The reason for this variation lies less in some undefined shock effect than in the compensation for the loss of one sensory mechanism by another.

Bigelow's observations on *Carassius* are in agreement with Parker's. The facility of maintenance of equilibrium when swimming slowly after loss of both internal ears was lost when the fish was made to swim rapidly in a large body of water. The effects are permanent.

Parker (35) found that the catfish *Amiurus* suffered loss of muscular tonus and the loss of the power of maintaining equilibrium after section of both eighth cranial nerves. Most of the time of such fishes is spent in lying on the bottom of the tank, often with the ventral side up. When they swim it is usually in a slow rolling way, and when driven to rapid motion, they swim in grotesque spirals.

Bethe (12) had previously found much the same effects in *Perea fluviatilis* and *Seardinius erythrophthalmus* after vestibular lesions. After a unilateral lesion, *Perea* shows a respiratory effect on the injured side. There is no loss of synchronism of the two sides in respiration, but the gill covers on the injured side are not raised as high as those on the uninjured side.

Ewald (36) confirmed on the eel, *Anguilla vulgaris*, the loss of strength noted by Gaglio in the dogfish. There were also severe disturbances of equilibrium.

*Batrachians.* Anatomical conditions in the batrachian ear preclude such exact and detailed study of individual parts of the vestibule as is possible in the sharks, but, on the other hand, the development of limbs makes possible the observation of the effects of vestibular lesions upon animals which have reached the second stage in the development of the vertebrate locomotor apparatus. There is also a further development of the central nervous system. By means of the limbs, these forms maintain a fixed position when at rest on the surface of some solid object, and hence are the first vertebrates which may be rotated on the turning table in such a way as to show the effect of vestibular excitation upon the resting position. The batrachians include also forms which, although they retain their gills throughout life and live in the water, effect their locomotion by means of limbs; other forms which retain their gills during the early part of life only and then emerge from the water upon the land, and still other forms which become so specialized that, after undergoing metamorphosis, their mode of progression upon the land is by hopping or jumping rather than by walking or crawling.

Stewart (37) used a perennibranchiate form *Necturus* (*Menobranchus*) for experiment. He found that destruction of the internal ear on one side was followed by rapid movements of rotation of the body,

always to the injured side, about its longitudinal axis. The movements are apparently involuntary and without fatigue. If for any reason these movements cease, they can be started again by excitation of the animal.

Ewald (38) found that in *Salamandra maculosa*, there are only slight motor symptoms as a result of vestibular lesions. After a unilateral lesion the legs of the opposite (uninjured) side are raised abnormally high, while those of the same side may be dragged along the ground.

Laudenbach (39) removed the entire vestibule, unilaterally and bilaterally, and the otoliths alone in *Siredon pisciformis*. After unilateral lesions of the whole vestibule, the animal rotates rapidly about its longitudinal axis when swimming, turning always toward the injured side. At first it can not go forward at all without undergoing this rotation. The disturbances are more marked in young animals than in old. Adults recover sufficiently in three or four days to swim slowly without turning over, but not to swim rapidly.

Whether bilateral operation was done on the same day, or whether the second labyrinth was removed after an interval, severe and permanent disturbances of orientation followed, when the animal was in the water. The animal maintained its position on the ground or at the bottom of the tank well. It could not maintain its normal position at the surface of the water, but floated belly upward with the nose under water. Any recovery that may have occurred after removal of one vestibule was lost after subsequent lesion of the second, so far as deportment in the water was concerned, and little recovery was seen even after fifteen months. The difficulty in swimming was so great that the animal could not get to the surface for air, and the gills again began to grow if the animal was kept permanently in the aquarium.

No noticeable disturbances of movement or equilibrium follow unilateral or bilateral removal of the otoliths without injury to the ampullae.

The effect of unilateral and bilateral lesions of the whole vestibule in the frog is so well known from the work of Schrader (40), Ewald and others as to require no special comment here. There is torsion of the head and body to the injured side after unilateral operation, swimming in a circle to the injured side and turning to this side in jumping. The fore limb of the sound side is strongly extended as if the animal were bracing itself by it, while the fore limb of the injured side is strongly flexed.

Henri (41) found that torsion of the head partially or almost completely disappears after an interval of six weeks or so. If decerebration is done at this time, the original symptoms reappear in all their original intensity, and no further amelioration of the conditions is observed so long as the animals live. If decerebration is done first and the vestibular operation afterward, there is no improvement in the condition of the animal as long as it lives.

Streeter's (42) observations on the effect of unilateral or bilateral removal of the auditory pit and acoustic ganglion in the tadpole are of considerable interest in relation to the ontogeny of vestibular function. The fact that it is possible to produce at will practically a congenital absence of the auditory vesicle serves as a control for the experiments on adult animals, and has a direct advantage in the permanence, completeness and lack of injury to contiguous structures over operations on adult forms. And since it may be presumed that the various organs possess their greatest degree of adaptability during the formative period, such embryonic interference affords a most complete test of the power of functional adaptation for loss of a specialized receptor.

The larval frog passes through three stages in acquiring the facility to swim, which Streeter names as follows; 1, The stage of non-motility—the first three days; 2, the stage of spinal reflexes—fourth to sixth days; 3, the stage of maintenance of equilibrium, sixth day to maturity. At the close of the first or non-motile stage, the structure which is to form the future auditory vesicle is visible and may be dissected out. The auditory vesicle never regenerates after its complete destruction, including the acoustic ganglion.

The larva makes no effort to swim in the first twenty-four hours, and it is only on the fourth day after the operation (about the sixth of larval life) that any significant difference in behavior is noticeable as a result of the lesion. At this time, when they, as well as the normals, are stirred up from their resting position on the bottom or against the sides of the dish, the operated individuals swim in spirals or circles. There is a tendency to swim with the injured side under, and the rolling movements which occur about the longitudinal axis are always directed toward the operated side. On reaching the bottom, they are able to direct their courses as the normals do. The characteristic disturbances in swimming become more marked in the next two days, but on the seventh day after operation many of the operated larvae may swim in a fairly direct course. On exciting them, the rolling

movements reappear. There is improvement from this time on, but the larvae always incline more or less to the injured side, and momentary loss of equilibrium is noticeable whenever they become excited.

Metamorphosis occurred at the end of the third month. No difference in attitude, whether at rest or in motion, could be made out in the operated as compared with the normal individuals after metamorphosis. Streeter thinks that the disappearance of the symptoms does not indicate any cure of the condition set up by the lesion, but that a slight defect is more difficult to recognize in the frog than in the tadpole. As a corollary, he points out that the maintenance of equilibrium in the tail-swimming tadpole is dependent upon a more delicately balanced mechanism than in the leg-swimming frog. It might be pointed out, also, that a more perfect set of peripheral effectors for the maintenance of equilibrium is present in the frog.

After bilateral operation, there was practically no difference to be made out in the appearance of the operated and the control larvae for the first three days. On the fourth day after the operation, they are decidedly less active than the controls, or than larvae with one auditory vesicle only removed. They make no swimming movements, but wriggle along on the bottom of the dish. Seven days after operation, they are smaller than one-sided or control specimens. They are symmetrical in form, but are less active and make only unsuccessful attempts at swimming. Twelve days after operation, any attempts at swimming resulted in a series of somersaults, after which they sank to the bottom in almost any position. There is great difficulty in feeding, and wriggling movements along the bottom are not as well executed as on the fourth and fifth days after operation. The larvae could not be carried much beyond two months of life, as they seemed unable to get food, and to go to the surface for air as normal larvae do. They were only about one third as large as the controls at this time, and had no more ability to swim than they had a few days after the operation.

One may remark that the severity of the effects of a bilateral lesion is much more than twice that of the effects of a unilateral lesion. This great difference in severity of effects seems difficult to account for on any other basis than the compensation for the loss of only one auditory vesicle by the other. And the larval form, while it compensates quite as perfectly as the adult for the loss of but one ear, is much less able to compensate for the loss of both than is the adult.

Laudenbach found no noticeable defects after removal of the otoliths only, with conservation of the ampullae, in the frog. Ach (43), however, reëxamined the question. He calls attention to Breuer's (26) analysis of the spatial relationships of the otoliths in the ear of the frog, and the general parallelism of each of them to the plane of some one canal. He confirmed Laudenbach's observations so far as any effects upon the attitude of the body or the usual movements of progression were concerned. Ach brought out, however, three effects of the bilateral removal of the otoliths. There are three reflexes,—the "Stirn" reflex, in which the frog assumes a position so that its body is concave toward the ground, supported only upon the tip of its nose and its hind feet; the shrieking reflex, which differs from the ordinary croak reflex; and the lid reflex of the eye. The first two are difficult or impossible of elicitation in normal frogs, while the lid reflex is usually present. After bilateral operation the first two, the "Stirn" reflex and the shrieking reflex, were much more easily elicited than before, while the lid reflex failed in progressive linear movements.

After unilateral lesions of the otoliths, the lid reflex is still elicitable at times. When the frog is moved up or down in a vertical direction, and particularly when it is moved downward, the lid reflex appears in the eye of the injured side. In linear movement from side to side, or backward and forward in the same plane, both eyes, or one only, may close, whether the otoliths are intact, or absent on one side. Ach never observed closure of the eye on the sound side only after unilateral operation. If only one eye closes when the otoliths of one side are absent, it is the eye opposite to that of the sound labyrinth. The relationship appears to be a crossed relationship. After bilateral removal of the otoliths, the lid reflex persists practically undiminished. *a*, when the frog is rotated in a horizontal plane about its dorsoventral axis; *b* upward or downward about a transverse axis; or *c*, from side to side about its longitudinal axis. Under conditions of rotation, the lid reflex must be due to excitation of the ampullae. He considers the failure of the lid reflex in progressive linear movements after bilateral removal of the otoliths as the most characteristic effect. He attempts to show that Breuer's idea of the perception of linear acceleration by the otoliths only is correct.

Graham Brown (44) investigated the effects of physiological excitation of the vestibule upon the respiratory movements of the frog. The effects of vestibular excitation are not peculiar, but are very similar to the effects arising from pinching the toes of one foot, and

apparently may all be comprised in the usual cycle of acceleration and augmentation of the respiratory movements. Bilateral removal of the otoliths does not affect the response to movement in a vertical direction, that is, to linear acceleration, but complete removal of both vestibules abolishes the response. Brown supposes that the ampullae may respond to movement in a straight line, and departs from Breuer's and Ach's views. Extirpation of both vestibules has no permanent effect upon the respiratory movements, although there is indication of some temporary loss of tonus of the muscles of the floor of the mouth.

*Reptiles.* The reptilian brain represents the highest type of generalized brain. The superficial layer of gray matter on the cerebral cortex has increased in amount, as compared with the batrachian brain (45) and there is some representation of the central terminations of the optic fibers in the cerebral hemispheres (46). In higher vertebrate phyla, there is development of the avian brain on the one hand characterized by the relative preponderance of the cerebellum, and the mammalian brain on the other hand, characterized by the relative preponderance of the cerebral hemispheres. The vestibule is enclosed in hard bone, so that sharply localized lesions of its various parts are not easy to effect, and the mode of reproduction renders the extirpation or transplantation of the embryonic vestibule more difficult than in some batrachians. The variations in body form, from the extremely elongated serpents without any limbs whatsoever, to the turtles with their extremely stable base of support for the body and long mobile neck, afford an opportunity for study of the effects of lesions of one peripheral sense organ upon various types of locomotion in one phylum, and upon various parts of the body as well.

Trendelenburg and Kühn (47) used the European swamp tortoise, *Emys lutaria*, for experiment. After unilateral operation, they observed torsion of the head to the injured side when the head was in the shell and bending of the head to the injured side when it was protruded. Slow progression on the land was not attended by any unusual disturbance. The torsion of the head and neck is permanent, but there is no turning to one side in progression. The swamp tortoise is more active in the water than on the land, and more marked disorders appear in swimming than in crawling. When at rest, the injured side is lower in the water, and in swimming there is movement in a circle to the injured side. Frequently the animal spins about a dorsoventral axis. It rights itself when placed back downward in the water.

Compensatory movements of the head are slower and eye movements more rapid in the turtle than in the lizard. The loss of one vestibule does not alter the reaction of the head to a change of position. If the visual field is kept stationary, both the rotation and the post-rotatory effects are obtained if the rotation is toward the injured side, but the after effect fails if the rotation is toward the sound side. This is a point of some interest, as it indicates that one vestibule has less capacity of response to rotation in either direction in these forms than it has in the human subject. If the visual field moves with the animal, the rotation effect fails when the rotation is toward the sound side.

Bilateral lesions are not attended by any unusual manifestation when the animal is at rest. The movements on land are slow, but not otherwise abnormal. The head moves back and forth without resistance when the animal is shaken. It rights itself when placed back downward on the ground, but may remain in the back downward position when swimming. It turns to one side or the other, and may even turn in a circle when swimming.

Van Rossem (48) had given, the year before, an exhaustive account of the vestibular symptoms in turtles. Our own observations (49) have been along the lines of the unilateral and bilateral extirpation of the vestibule and electrical stimulation of the ear. Our results on extirpation are in general agreement with those of Trendelenburg and Kühn. Along with the torsion of the head following unilateral operation, there is a displacement of the eyes such that the eye of the injured side is turned upward and the other downward. When floating at rest, the limbs of the injured side are flexed closer to the body while those of the opposite side are extended. Food is grasped accurately and securely after the first few days. The torsion of the head lasts as long as the turtle lives, eight months in the case of some specimens. Any decrease in the torsion occurring in the first months of recovery is lost after decerebration. The torsion may become even more marked than before.

The effects of bilateral operation are more severe. The animal floats level in the water, and *Nanemys* will float on its back without righting itself. *Chrysemys*, because of the peculiar shape of its body, will not remain on its back in the water at all. In swimming, *Nanemys* may go in a small circle, swimming on its side with the back directed toward the center, or it may spin about its dorsoventral axis and stop with all limbs strongly extended and its nose pointing almost straight upward.

Control specimens grasped pieces of fish placed in the enclosure readily and accurately. The body is held immovable and the neck and head are suddenly thrust out. The jaws close with a snap as the food is reached, and the head is quickly retracted. I have seen a turtle, *Chrysemys*, repeatedly grasp at food after bilateral operation, without ever touching it. The head was retracted after each failure and again thrust out, only to go to one side or the other or above or below the desired morsel. Although evidently hungry, the animal might finally give up the attempt to grasp the food and crawl away. The broad flat body of the turtle affords a secure basis of support, and the inability to grasp the food signifies that the animal is unable, even with the aid of its eyes and muscular sense, to control the rapid movements of the head accurately. These erratic movements of the head bring out sharply the importance of Crum Brown's statement that the function of the vestibule is to give information of the aspect or change of aspect of the head in space. The vestibule is a proprioceptive structure whose biologically adequate stimulus arises from the aspect or change of aspect of the head in space, from the aspect or change of aspect of no other part of the body in space. Its loss leads to centripetal ataxia.

Electrical stimulation of the ear of the turtle is easily accomplished (50). When the direct current from fifteen to twenty dry cells is passed through the head from one ear to the other, there is a movement of the body, brought about largely by the hind feet, so that the head is turned toward the side of the anode. The response persists after decerebration.

Loeb (51) brought out the important principle in the horned toad *Phrynosoma*, of the algebraic summation of optical and vestibular stimuli or, as he calls them, heliotropic and geotropic effects in the elicitation of compensatory movements during and after rotation. When the lizard is rotated, with ears intact and eyes closed, a speed may be found which will elicit barely perceptible compensatory movements of the head during rotation. But on suddenly stopping the rotation the animal will exhibit very marked compensatory movements. With the eyes open the effects are just the reverse. Two animals, one with the eyes open and the other with the eyes closed, may be placed on the same turning table at the same time, and these differences in deportment noted under exactly the same conditions of speed of rotation and suddenness of stopping. If the visual field is rotated at the same speed as the animal, the effects with the eyes open are the same as with the eyes closed. If an endless roll of paper with wide vertical lines marked

on it is rotated about the animal, there are compensatory movements of the head during the movements of the paper, but none after the paper has ceased to move.

The fact that the effect of rotation may persist in animals—frog (Ewald), turtle (Trendelenburg and Kühn) after bilateral lesions of the vestibule when the eyes are open but not when the eyes are closed had been known for some time previously, and the neglect of precautionary measures has led to some incorrect statements. The fact that, in *Phrynosoma*, the most marked compensatory movements of the head appear after rotation has ceased when the eyes are closed seems to dispose effectively of Schäfer's (52) analogy of the loosely jointed wooden head and his view that the compensatory movements of the head during rotation arise from the inertia of the head itself. Gruenberg (53) had concluded from experiments on the frog that the "spin" was the only constant element in the excitation of the vestibule during rotation, and Maxwell (54) showed on *Phrynosoma* that the torsion effect was the essential element. The two names appear to indicate exactly the same thing.

Trendelenburg and Kühn found that, after bilateral lesions of the vestibule, the common lizard, *Lacerta agilis*, showed no abnormal position of the head when at rest. From time to time, however, swaying of the head from side to side would appear. These movements were especially noticeable after the animal had been making rather vigorous movements of the body and had come to rest. The animals had great difficulty in catching the meal worms on which they were fed, the head swaying about so much that it was largely a matter of chance when a worm was caught.

Henri (55) considered it of interest from the theoretical point of view to investigate the effects of vestibular lesions in serpents because of their peculiar method of progression. The effects of vestibular lesions in the adder are manifested in various ways. There is a general feebleness and sluggishness of movement. The intact snake raises its head slightly above the ground in crawling, but, after operation, it rests or scrapes its lower jaw on the ground, and there is a tendency to turn to the injured side after unilateral operation. If held by the middle of the body, such an animal attempts to raise its head to reach the sustaining hand, but, in so doing, it turns its head around in a circle and rotates about the long axis of its body. The head is lower on the injured side when crawling or at rest. It may even rotate about its long axis in crawling. If placed on its back, it rights itself, turning always

to the injured side. Movements of the head may persist for some time after crawling has ceased. These observations were confirmed by Trendelenburg and Kühn. They found that the rolling movements of the body occur only when the snake is on a smooth surface, and not on a rough.

After bilateral operation, the position when at rest differs in no noticeable respect from the normal. But uncertainty is apparent as soon as the snake attempts to move. The head moves back and forth like a pendulum, with only momentary interruptions. If the snake is grasped by the anterior part of the body and shaken, the head moves aimlessly back and forth. In a normal animal the head moves along with the body. In swimming the head sways from side to side with the movements of the body instead of maintaining its median position, as in the normal specimen. Spiral bending and swimming occur at times. The back upward position in the water is very stable, even when the eyes are closed with collodion. Only when dropped from a height does the snake turn ventral side up in the water.

Most of the actual information derived from a experimental source as to the function of various parts of the vestibule and the mechanism of excitation has come from the study of the representatives of the three lower phyla of vertebrates. The rôle of the receptor in giving the animal control of the movements of the head is clear from a study of the experimental results. The difference in effect of unilateral or bilateral lesions upon various closely related forms with different methods of progression, but all of which are alike in possessing a central nervous system which does not show any very high degree of development, as compared with the mammalian type, and which vary from forms which spend their whole life in the water to those which enter the water only incidentally, are better shown here than in any other animal types used for experiment. One finds all variations from no receptor whose normal mechanism of excitation depends upon the aspect or change of aspect of the head in space, and no special effectors for correcting any variation of the head or body from the normal position, to a specialized receptor and a complete equipment of effectors. In between these extremes there are rudimentary or primitive effectors, or even their total absence. The eel and the serpent have no special effectors aside from the general musculature of the body. There are no paired appendages. But the presence of the specialized receptor enables the animal so to manipulate or control its general body musculature that all variations of the body and head from the normal position may be

remedied promptly and effectively. Similarly, Siredon may suffer the loss of all four limbs but, as long as the vestibular endings are intact, the animal suffers no loss of motor control in the water. One need go no further than the forms already described to find ample reason for doubting Ayers' (2) pessimism as to why the vestibular mechanism should have been evolved.

Passing from these lower vertebrates with the generalized nervous system to the birds and mammals, the nature of the problem changes somewhat. The increasing efficiency of the central nervous system as an integrating mechanism, and the increasing complexity, with a possible increase in its modalities, of a peripheral sensory mechanism shift the emphasis from the effects of the excitation or lesion of one peripheral sensory structure as we find it in lower vertebrates, to the ability of a more developed central integrating mechanism to compensate for and largely mask the effects of the loss of a single peripheral receptor. The development of the exteroceptors, and particularly of the distance receptors, in the land-living mammals and birds introduces another element. The free, powerful swimming fishes are dependent upon proprioceptors alone for the maintenance or control of the position of the body while in the water and not in contact with the bottom or other solid or motionless objects. There are few fixed points of reference in their environment of which they take cognizance through the exteroceptors, contact or distance. Probably there are few fixed points in the open ocean through or by which a whale orients itself, but with the other land-dwelling mammals, and particularly man, the case is different. There are few times in the life of an ordinary mammal on land when it is not in contact with the earth or some other fixed object. In the water or in the air, the case may be a little different, and the study of the effects of vestibular lesions in birds and mammals should be carried out under conditions as similar as possible to those under which the vestibular mechanism is of greatest usefulness in the lower vertebrates, that is, when an animal is prevented from coming in contact with fixed objects in the environment, or when, through elimination of vision, it is prevented from orienting itself through the visual exteroceptors.

It is to be presumed that the effects of vestibular lesions in lower vertebrates are more nearly objective than subjective. In the human, subjective effects appear, such as vertigo and the general group of sensations which have been included under the term sensation of rotation or of apparent movement. There is presumably an organic basis for

such sensations, but their analysis leads one into the central nervous system rather than to mere lesions of peripheral receptors. A fundamental principle in the interpretation of the effects of central nervous lesions in different animal forms enters in here,—the principle of the migration of function toward the anterior or cephalic portion of the central nervous axis (46).

*Birds.* The vestibule of birds has, from the time of Flourens to the present, held a central place in the study of its function. The work of Flourens on the experimental elimination of the internal ear marked the first stage in the experimental study of this receptor. The second stage began when Goltz (21) formulated his theory that the semi-circular canals were sense organs for the perception of the position of the head in space. He proposed a hydrostatic theory of stimulation of the vestibule through changes in hydrostatic pressure of the fluid with the changing position of the head in space. The hydrostatic theory fell into disuse after the development of the dynamic theory of Mach, Breuer and Crum Brown. There is abundant evidence that the dynamic theory is applicable under many conditions of animal activity, but to discard totally the hydrostatic theory at present seems premature. We have only to remember that the change in the position of the eyes of a normal dogfish lasts as long as the head is held in the changed position, long after any inertia of the fluid in the vestibule has been overcome, to see one possible application of the hydrostatic theory. That it is not necessarily due to displacement of the otoliths is shown by the fact that the same displacement of the eye occurs after removal of the otoliths.

Ewald (56) called attention to the state of the vestibular problem at that time in the introduction to his first paper on the function of the vestibule in birds. Experiments up to that time had yielded, as they have up to the present, conflicting results. The effects of vestibular lesions in birds, up to that time, had been more severe and more permanent than the effects of similar lesions in fishes or mammals. The most reliable work on fishes has appeared since 1887, and the effects of vestibular lesions in birds have gained enormously in clearness, accuracy and fulness of detail from the work of Ewald himself. Better methods of operation have been devised in mammals, and the observations of the effects of lesions have been more carefully made since that time, but the differences in effect which Ewald noted are still more or less apparent. The immediate effects of operation are very similar in severity in all types of vertebrates which have come under my

observation, but the remote effects differ in intensity, and particularly under varying conditions. The effects of bilateral lesions in larval frogs are more severe than the effects of similar lesions in birds or mammals. The severity and permanency of the effects in Siredon are fully equal to those in birds, and more than equal to the effects observed in mammals under ordinary conditions. Ewald's statement, therefore, needs some revision in view of the greater number of facts now available on a greater range of animal forms.

Ewald stated three possibilities, one or more of which might account for this difference in the effect of vestibular lesions: 1, There may be a difference in the topographical relations of the vestibule in different types of animals, so that contiguous structures may be damaged in different degrees in vestibular operations on various animals. For example, neighboring regions of the brain may be damaged more in birds, less in mammals, and not at all in fishes. It was not until Lange (57) showed that the effects of vestibular lesions in birds were not dependent in any degree upon injury to the cerebellum that this question was answered for birds. And no good experimenter need inflict any injury whatsoever upon the central nervous system of any animal in operating upon the vestibule. 2, We may say that the vestibule has different functions, or at least different degrees of the same function, in widely separated groups of vertebrates. Fish may have a rudimentary development of the vestibular sense, mammals a somewhat better development, and birds the highest development of all. By way of comment on this possibility, it may be said that we have no index of the acuity of the vestibular endings in any vertebrate except man. It is possible, of course, that the acuity of the vestibular sense, to follow Ewald's terminology, may vary in different animal forms just as the acuity and sharpness of vision or the acuity of smell may vary, even in different individuals of the same species. 3, We may say that the vestibule has the same function in different animal forms, and that, following its destruction, the same functional defects appear in all of them; but these defects may be more or less, possibly completely, obscured by the static conditions under which the animal lives. Under these static conditions are included the general structure of the body, the length of limbs, the length of the neck, the number and size of the points of support, the topography and size of the supporting surface, in relation to the form of the body and the medium in which the animal lives. These considerations are important. As I have mentioned above, the animals living on the land are, under all ordinary circum-

stances, in contact with a solid surface, that of the earth itself, only occasionally, depending largely upon habit, going into the water or into the air. Birds go into an even more unstable medium than water,—the air. It seems to me that we should now add at least one more consideration, 4, that of the degree of development of the exteroceptive sense organs, particularly the distance receptors, and the degree of development of the central nervous system itself.

In order to test out these various possibilities, Ewald made a series of experiments on various representatives of a single phylum and chose birds. In this phylum, if one does not choose genera too remote from each other, the topography of the vestibule is too similar to permit of many differences in effect arising from varying degrees of injury to contiguous structures, and one would think still less of a difference in function of the vestibule in different families of the same phylum. On the other hand, the static conditions in different families and genera of birds vary greatly according to whether they run, jump, fly or swim. The mode of station at rest also varies, as some lie, sit, stand on two legs or on one. Such a study should bring out the extent to which the functional disturbances following lesions of the vestibule are dependent upon the static conditions of the birds chosen for experiment. Ewald's results are given in the accompanying table.

Ewald emphasizes the fact that the difficulty in flying is more marked in birds whose wing movements are more rapid than in those birds whose wing movements are slower. The swallow had the most highly developed powers of flight of any of the forms used for experiment, and the disturbances were greatest in this bird. He draws the general conclusion that when one makes the same destructive lesion of the vestibule in each of a series of different forms of birds, the resulting disturbances of movement are greater the more difficult it is for the bird to maintain its equilibrium normally in one form of progression, and the greater or more rapid the degree of muscular coördination required for its successful execution.

Experimental work has been carried out in birds, and particularly in pigeons, in much greater detail. In general, there is torsion of the head to the injured side, sometimes leaning of the body to that side, inequalities of wing movements, the movements being less extensive and of less force on the injured side, and a loss of general muscular tonus on one side, when the lesion is unilateral. Rotation is attended by head movements, head nystagmus, as Breuer calls it, and ocular nystagmus, in normal birds. The reactions to rotation are lost after bilateral

vestibular lesions when the eyes are covered. There is also a great disturbance in the sense of position of the head, and Ewald argues for a permanent loss of tonus of the neck muscles.

Bornhardt (58) applied ice to the left posterior vertical canal of a pigeon and observed rapid movements of the eyelids, the eyes being closed; when the pigeon attempted to move, there were rather feeble backward movements of the head and traces of circus motion. Continued cooling of both posterior vertical canals increased the backward movements of the head and the tendency to fall backward. Pendulum

TABLE 1

DISTURBANCE	FLYING	JUMPING	HOPPING	WALKING	SWIMMING	STANDING
Very marked	Swallow					
Marked	Sparrow	Raven				
Moderate	Pigeon Raven	Sparrow	Raven Sparrow			
Slight	Hen			Raven Pigeon Hen		
Transitory	Goose			Goose	Goose	Raven Hen Sparrow Pigeon Goose

movements of the head, occasionally passing over into circus movements of the body, also appeared. All effects disappeared in fifteen minutes after cooling was intermitted. The application of a hot rod to the left horizontal canal of a pigeon caused movement of the head to the right and closure of the eyelids. When the eyelids were opened there was strong deviation of the eyes to the right, with quick jerks to the left. Burning the right horizontal canal of the same bird caused movements of the head to the left, but less marked than when it had before turned to the right, and it soon returned to the midline.

Considerable work has been done on mechanical stimulation of various separate canals in the pigeon. Ewald (38), by means of his pneumatic hammer, set up pressure in a canal which was transmitted to its ampulla. There was, in the case of the horizontal canal, a movement of the head

to the opposite side in the plane of the canal. There is usually a return to the normal position immediately afterward. When the pressure is relieved, there is a movement of the head toward the side stimulated, but less marked than when the pressure was increased. In the case of the superior (vertical) canals, the movement following the relief of pressure is more marked than the movement following the increase of pressure. There is also movement of both eyes, the direction of the movement being the same in the pigeon as in the dogfish.

It should be pointed out that Maxwell's result on the transmission of pressure from the utricle to the ampullae of the various canals is not in any way inconsistent with Ewald's results. The facts seem to indicate that, wherever the pressure may arise, whether in the canals or in the utricle, its transmission to the ampullae is sufficient to excite the nerve endings within them. This is the important thing to be remembered in formulating any hypothesis of the normal mechanism of excitation of the ampullae.

Breuer (31) has repeated, on pigeons, Gaglio's (29) experiments on the application of cocaine to the vestibule. There is an agreement that simple anesthesia of the vestibule is attended by the same effects as its anatomical destruction. Breuer, however, disagrees with Gaglio in some matters of detail.

Borries (59), basing his conclusions partly upon experimental results in pigeons and partly upon clinical observations, thinks that the changes in the reaction to irrigation of the ears with water colder or warmer than the body,—the caloric nystagmus of the clinician,—with the changes in the position of the head are dependent upon the otoliths. I am unable to follow the reasoning involved in arriving at such a conclusion. While it has been shown by various experiments that we must probably attach more importance to the otoliths than we have heretofore, it has not yet been shown that the ampullae of the vestibule are without any functional importance. It seems much more likely that we must regard both parts of the vestibule as functioning together to bring about a common reaction.

*Mammals.* Although the first experimental observations on the effects of excitation of the vestibule were made by Darwin and Purkinje, perhaps even by Theophrastus, on the human subject, the greatest knowledge of the functions and manner of excitation of the vestibule has not been derived from observations upon the human or even upon mammals. As has been indicated, the nature of the problem changes somewhat when one reaches the birds and particularly the mammals.

Vertigo, with which Purkinje dealt, is a subjective thing and not wholly objective. Its consideration goes far beyond the mere function of the vestibule and leads to a problem of extreme complexity in the central nervous system itself. Purkinje's results have been so well presented elsewhere (60) that their consideration here is superfluous, and the problems of the central nervous system are much too complex to enter upon here. Raines (61) has given a specific instance of the phenomena of vertigo appearing under certain conditions in aviators.

The ocular nystagmus, mentioned in birds, is a prominent feature of the effects of rotation in mammals. The detailed description of all forms of ocular nystagmus is given by Coppez (62). The typical form of vestibular nystagmus, as observed in man and the higher mammals, is not elicited by lesions of any other nervous mechanism, so far as my observation or knowledge goes, and it does not appear in typical form in lower vertebrates. Its consideration becomes not so much a question of vestibular function as a problem of the connections of the vestibular and oculomotor nerves within the central nervous system. As Eckhard (63) states, when it is certain that no injury to the medulla oblongata occurs, there is no ocular nystagmus following lesions of the cerebellum. Our original statement that complete removal of the cerebellum in the dog does not prevent the appearance of vestibular nystagmus has now been confirmed by Magnus for other mammals.

Hitzig (64) showed that exactly the same phenomena of vertigo could be produced by electrical stimulation of the vestibule as result from rotation of an animal or man. The clinical application of the electrical method of stimulation of the vestibule has been considered by Babinski (65) and others.

All mammals, except man, show the torsion of the head to the injured side after unilateral vestibular lesions, with rolling movements to that side during the first few days. The torsion of the head is permanent, but the rolling movements and the nystagmus are transitory. The same effects are often seen accompanying abscesses of the inner ear in white mice and rats (66), guinea pigs and rabbits. I have never seen a case of an abscess of the internal ear in cats or dogs. Walking or running in a circle to the injured side is also a transitory effect of a unilateral vestibular lesion.

After bilateral operation there is a slow swaying of the head from side to side, especially in cats, while dogs remain flat on the floor for some days afterward, refusing to walk or even stand. The decerebrate animal will stand, even after removal of both vestibules (67). Walking

or running in a straight line soon becomes possible, and dogs or cats exhibit no noticeable defects of locomotion when on the ground. When the eyes are covered, the dog has difficulty in walking in a straight line, turning from side to side in an irregular way. Swimming with eyes open is a matter of great difficulty or even impossible in such an animal. It may swim on its side or back or turn downward toward the bottom of the pool. After unilateral operation a cat or dog will jump over the side of the cage or from a considerable height to the ground without falling or exhibiting any defects of movement. But a dog will not jump from even a moderate height to the floor without falling in a heap after bilateral operation. This observation was made by Schiff (68), and we have many times confirmed it. After one or two experiences, a dog will look over the edge of the table and whimper when food is held some distance from him, but he may refuse to jump to the floor in order to get it. Under conditions where the animal is deprived of the use of its proprioceptors and exteroceptors with the exception of sight, as in jumping or swimming, the effects of vestibular operations may be shown in dogs months afterward.

The deviation of the eyes is permanent in lower vertebrates following unilateral vestibular lesions, but it is merely transitory in higher forms. It has been found, also, that the nystagmus following rotation may be decreased or even abolished by repeated rotation of an animal or man on successive days (69), (70), (71). And with reduction of nystagmus there is a reduction in the degree of vertigo experienced in men. This does not seem to indicate any particular difference in the function of the vestibule of higher forms as compared with the lower, but to indicate that there is a considerable difference in the central nervous relations of the vestibular and oculomotor mechanism in higher forms. The problem again becomes one of the organization of the central system and its changes in phylogenetic development. And no place outside of the vestibule can one find any part of a proprioceptive mechanism which is circumscribed in location and which is capable of complete anatomical removal with so little damage to any contiguous nervous structures. For this reason as well as because of the very definite motor and postural disturbances following anatomical lesions the vestibular nerve endings offer a unique opportunity for the analysis of the central relationships of an extremely important proprioceptor. The problem is as yet unsolved.

Dodge (72) has made the only determinations of the threshold of stimulation of the vestibular endings known to me. The threshold

lies between  $1^{\circ}$  and  $2^{\circ}$  per second for sine curve acceleration beginning at zero. During actual rotation the threshold seems to be higher, as a change of  $6^{\circ}$  a second seems necessary before the subject notices any change in the rate of rotation.

Aside from the ordinary results of rotation, some other facts of interest have been noted in connection with the mammalian vestibule. Magnus and de Kleijn (73) showed that when a cat's head was lowered, the vestibules being intact, the fore legs were flexed and the hind limbs extended; when the head was raised, the fore limbs were extended and the hind limbs flexed. The numerous other papers of these authors are given in Griffith's (11) bibliography.

Wilson (74) has shown that high explosives may so affect the cochlear portion of the ear that hearing is impossible, but the vestibule may remain relatively uninjured. The history of the separation of the auditory and vestibular functions of the internal ear is given by Gellé (75). Another extremely important series of observations is that of Holmes (76) on the effect of gunshot injuries of the cerebellum. As Holmes points out, the effects of gunshot injuries in the human are more nearly comparable with the experimental results in animals than are the effects of ordinary pathological lesions.

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## ADDENDUM

The two following important publications were received too late for comment in the text.

- MAXWELL, S. S. *Labyrinth and equilibrium*. Philadelphia and London, 1923.
- MUSKENS, L. J. J. *Brain*, 1922-23, xlv, 454.

## ANTI-ANAPHYLAXIS AND DESENSITIZATION

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The recent reviews by Arthurs (1), Bordet (2), Coca (3), Dale (4), Wells (5), Zinsser (6) and particularly the extensive articles by Doerr (7) have presented the subject of anaphylaxis in so many of its phases and so satisfactorily that it seems unnecessary at this time, and is obviously impossible in the space devoted to this article, to repeat what has already been so thoroughly discussed in these papers, most of which are readily accessible. As the outline by Wells that appeared in the *PHYSIOLOGICAL REVIEWS* for January, 1921, gives in an admirable way the general knowledge of anaphylaxis as it has been developed experimentally, therefore an attempt will be made in this review only to elaborate one or two phases of the subject that have not been so exhaustively discussed in these articles.

The recognition of states of sensitization in the human being that simulate or are analogous to the anaphylactic state in the animal has, for some years, attracted attention, and a considerable amount of work has been done in an endeavor to determine what diseases owe their origin to this condition of hypersensitiveness. Through these studies it has been well established that in many patients hay fever, asthma, acute intestinal disturbances occurring particularly in children, various forms of skin affections, such as eczema, urticaria and different types of dermatitis and occasionally angioneurotic edema, depend upon the fact that the individual possesses an abnormal hypersensitiveness toward some substance, often, but not invariably, containing foreign proteins. The most common of these are the pollens of plants, the dander and hair of animals, the feathers of birds, food substances, and even drugs and chemicals. It seems clearly proven now that there are direct relationships between hay fever and the inhalation of certain varieties of pollen, between the perennial forms of rhinitis and asthma and the inhalation of the dander of animals, the dust from feathers, the powder of roots, such as orris, or the ingestion of foods such as eggs, milk and cereal. A direct association has further been established between the appearance in certain individuals of eczema, urticaria,

different forms of dermatitis and angioneurotic edema and the contact or ingestion of many of the substances already mentioned or even of chemicals. In an attempt to relieve the individual of the affection from which he has been suffering, methods have been employed to reduce or to abolish the state of hypersensitiveness which is known to form the basis of these reactions, and it is particularly with the subject of anti-anaphylaxis or desensitization that this review will deal.

The original observations upon which all subsequent efforts to bring about suppression of the state of anaphylaxis by special methods have been based were made by Besredka and Steinhart (8), confirmed almost immediately by Otto, Rosenau and Anderson and Gay and Southard, and developed experimentally especially by Besredka and Weil.

With the object of finding some method that might be applicable in preventing anaphylactic shock and serum disease arising in man after the use of horse serum for therapeutic purposes, Besredka (9) experimented with a great number of substances, which, it was hoped, might counteract the effect of the hypothetical poison that was at this time supposed to induce the anaphylactic shock. Among other methods he tried the effect which heat had on the horse serum that was employed for the second or intoxicating dose of protein injected into the sensitized guinea pig. This disclosed the fact that heat impaired materially the activity of serum employed for the second injection. Horse serum, diluted in the proportion of 1 to 4 with distilled water to prevent coagulation, he found could be heated to 76°C. for 20 minutes without materially affecting its activity. Kept at a temperature of 89°C. for 20 minutes, some decrease in activity was observed. This impairment was more evident when the diluted serum was kept at 95°C. for the same length of time, and at a temperature of 100°C. for 20 minutes the activity of the serum was completely abolished. Provided the horse serum was heated, many times the fatal dose could be injected intravenously in highly sensitized guinea pigs without causing untoward symptoms. Besredka further observed that though the injection of heated serum might be harmless, it possessed the power of protecting the guinea for some time against injections of unheated serum, which, in themselves, were surely fatal. Further experiments demonstrated that the same effect could be obtained with the specific unheated serum when this was injected subcutaneously, intraperitoneally, intrathecally, or intravenously in amounts considerably below the minimal lethal dose. Sensitized guinea pigs could thus be protected against several times the lethal dose of horse serum by a single injection that was

200 to 500 times below the lethal dose. This refractory phase which Besredka first called anti-anaphylaxis was later designated by Weil, and indeed Besredka himself, as desensitization, a term which describes much more appropriately than anti-anaphylaxis this particular condition. It will therefore be referred to in this article as desensitization.

Thomsen (10) has stated very clearly the theories that may be offered to explain desensitization. He suggests that this may occur in at least three ways:

I. By the destruction or exhaustion of the antibody (occasioned by the preliminary injection).

II. By the interference with the reaction between antigen and antibody.

III. By the insusceptibility or diminished activity of the animal toward the shock-producing factor, which is an hypothetical and indefinable substance.

It may be seen that these explanations assume first that an antibody is formed toward the specific protein which is introduced parenterally; and secondly, that the symptoms of anaphylactic shock are in some manner associated with the union of the specific antigen and antibody. It is not possible in the present article to bring forward all the evidence in support of this view, but it is sufficient to say that though investigators have differed in their opinion as to the situation and the manner of the union between antigen and antibody, and though the intermediate steps between this union and the actual evidences of anaphylactic shock are still very obscure, still there is general agreement that the symptoms, which result from the injection of a specific protein in a sensitized animal are, in some way, dependent upon the interaction of antigen and antibody.

To explain the protective effect of a single sublethal dose of specific protein against a lethal dose in a sensitized guinea pig, Besredka advanced the hypothesis that the protein given in the preliminary injection, united with the antibodies and by doing so reduced their potency or combining power. This resulted in a condition which necessitated the injection of much greater amounts of antigen in subsequent injections in order to bring about an explosive reaction with death. His experiments seemed to show, moreover, that it was possible to increase, almost to an indefinite point, the tolerance of sensitized guinea pigs toward the specific protein by repeating, at short intervals and in increasing amounts, injections of the specific protein. Thus a sensitized guinea pig, for which an intravenous dose of 0.05 cc. of serum would be fatal

was given 0.025 cc. of serum intravenously without harm; 5 minutes later 0.10 cc. without serious symptoms; 2 minutes later 0.25 cc. without symptoms, and 2 minutes later 1.0 cc. could be administered, twenty times the lethal dose, without harm. This method of desensitization could be employed in passively sensitized animals as well as in actively sensitized animals. The preliminary injections were efficacious when administered by vein, intracerebrally, intraperitoneally or subcutaneously. Larger doses were necessary for desensitization when the injections were made intraperitoneally or subcutaneously, and required a much longer time to take effect than when administered intravenously. The fact that a rapid union of antigen and antibody is necessary for the production of the explosive reaction that characterizes anaphylactic shock, led Friedberger and Mita (11) to employ a method of desensitization which varied in technique from that employed by Besredka. They showed that when horse serum highly diluted with salt solution was infused at an extremely slow rate into sensitized guinea pigs, desensitization could eventually be established, and that guinea pigs treated in this manner were refractory to large doses of horse serum.

In general these observations have been confirmed by Friedberger and Mita, Weil (12) and many subsequent investigators; but not infrequently such irregularities occur in the results that have been obtained during the course of desensitization, that the correctness of Besredka's view has been called in question. Thomsen, who has experimented most carefully on this subject, emphasizes the fact that the rate and degree of sensitization varies considerably in different groups of guinea pigs, is influenced by the age of the animal and is affected materially by the size of the sensitizing dose. It is well known that small doses of serum sensitize guinea pigs more rapidly than large doses. In Thomsen's experiments an injection of 0.004 cc. of horse serum, in one group of guinea pigs, resulted in increasing sensitiveness up to the 25th day; after this time the degree of sensitization gradually diminished, but was still present 285 days after the original injection. When the sensitizing dose was larger, 0.1 cc., the height of sensitization was not reached until the 62nd day after injection; but, on the other hand, persisted for at least 365 days. He further confirmed Friedberger and Simmel (13) in the view that on the one hand very young guinea pigs and on the other old guinea pigs are much more resistant both to active and passive sensitization than are guinea pigs which weigh from 200 to 600 grams. It is obviously of great importance to keep these facts in mind when considering the results of any method of desensitization,

for Thomsen shows quite clearly in his experiments, that it is much more difficult to desensitize guinea pigs that are highly sensitized, than those whose sensitization is of only moderate degree. With high grades of sensitization one-half the m.l.d. of horse serum injected intravenously increased the resistance of the guinea pig very slightly, was perceptible in  $2\frac{1}{2}$  hours and persisted for 8 days; whereas with moderate grades of sensitization a single intravenous injection of one-half the m.l.d. increased the resistance of the guinea pig 30 times, was partially effective in 20 minutes, completely effective in  $2\frac{1}{2}$  hours and persisted 8 days. The same quantitative relation between the degree of sensitization and the relative difficulty of desensitization held when the guinea pigs were sensitized passively, for guinea pigs, made passively sensitive by the injection of small amounts of antihorse rabbit serum, could be much more readily desensitized to horse serum than those injected with large quantities. Thomsen's experiments seem to show that there is a close relationship between the amounts of antibody in the sensitized guinea pig and the relative difficulty of desensitization, the presence of large amounts of antibody rendering desensitization difficult; and he concludes that during the process of desensitization antibody disappears.

It seems quite clear also from the work of Dale and his co-workers (14), Weil (15) and others, that it is the tissue cells which are exhausted of their antibody during this process of desensitization. Dale, who has developed so extensively the use of the cornu of the uterus in experiments upon anaphylaxis, showed that the entire process of desensitization could be carried out *in vitro*; for, if a guinea pig is sensitized to two different proteins, the addition of one specific protein to the Ringer's solution, in which the uterine segment is suspended, renders the uterus insensitive to subsequent application of this specific protein, but leaves the reactivity of the uterus unimpaired toward the second protein to which the guinea pig was sensitive. The experiments of Dale and his co-workers are of great importance in demonstrating the fact that the mechanism of specific desensitization affects the tissue cells, in both actively and passively sensitized guinea pigs, and further throw light upon the condition of the cells during their phase of desensitization. One may ask whether the cell during this period is restored to a condition which approximates normal, and is devoid of all substances that might react either with antigen or antibody. Weil (16) has made some important contributions to this subject, for he has shown that when a guinea pig, which has been sensitized to horse serum, is desensitized by

repeated injections of horse serum, the animal may, during this period of desensitization; be passively sensitized to horse serum with the same facility as a control normal guinea pig. These experiments would indicate that, during the phase of desensitization, not only is the antibody completely dispensed with, but the cells return to a state which so nearly approximates normal that they are susceptible of passive sensitization to the same protein.

Such experiments have been carried out not only *in vivo* but *in vitro*, as Dale has demonstrated by employing the strips of uterine muscle. In Dale's (17) experiments the uterus of a guinea pig, passively sensitized to horse serum, was removed, suspended in Ringer's solution and treated with horse serum. The reaction which followed the first application could not be obtained again by applications made some minutes later. This indicated that the uterine muscle had been desensitized. After thorough washing, the muscle was soaked for  $2\frac{1}{2}$  hours in 10 per cent dilution of serum from guinea pigs anaphylactic to horse serum, and then washed in numerous changes of Ringer's solution. The response to a further addition of horse serum showed that passive sensitiveness had again been conferred upon the muscle *in vitro*, and could again be removed by an effective dose of antigen, since further additions of horse serum had no effect.

It is to be noted that, during the process of specific desensitization, the affinity or receptivity of the antibody for the antigen gradually diminishes; though in the partially desensitized animal such an affinity may still persist. In the partially desensitized state, however, much larger doses of antigen are required to produce a reaction than before desensitization has been started. As Weil (18) has pointed out, the quantitative relationships between the supposed union of antigen and antibody differ from those observed between precipitin and precipitinogen, and resemble more closely the phenomena encountered during toxin antitoxin neutralization when small quantities of antitoxin are added to toxin.

These experiments, which have been purposely set forth in some detail, all lead to the conclusion that specific desensitization of both actively and passively sensitized guinea pigs is brought about by the neutralization or exhaustion of the specific antibodies. This conception of the process has been adopted by most authorities, and by many the view is accepted that the process of desensitization takes place within the cells of the body.

Before considering the application of these methods of specific desensitization to therapeutics, it is necessary to call attention to another form of insusceptibility to anaphylactic shock, that was observed by Rosenau and Anderson to follow the repeated injection, at short intervals, of foreign protein in animals. Guinea pigs treated by this method are found to be highly insusceptible to anaphylactic shock. At the same time they contain specific antibodies circulating free in the blood, so that their serum, when injected into a normal guinea pig, is capable of sensitizing this normal animal passively to the specific protein. In rabbits treated by this manner the serum not only contains large amounts of antibody or anaphylactin, but is very rich in specific precipitins; and, as Doerr and Russ (19) have shown, there is a very close quantitative relationship between the precipitin content and the anaphylactin content of the serum of these immunized rabbits. The protection which these animals possess against injections of antigen seems to reside in the presence of these circulating antibodies, which unite with the antigen and prevent it from injuring the cells of the body. When these antibodies are removed from the circulation, as has been shown by Weil (20), the guinea pig becomes highly susceptible to injections of antigen. Likewise, when the organs are deprived of this protection they show *in vitro* the reactions that are characteristic of the highly sensitized animals. The experiments of Dale and of Manwaring and Kusama (21) show that the organs removed from immunized guinea pigs, and washed free from all circulating antibodies, react in the same manner to the specific antigen as the organs from highly sensitized guinea pigs, and these experiments add convincing evidence for the conclusion that the protection of these animals resides in the presence of free antibody in the circulating blood.

**SPECIFIC DESENSITIZATION OF HUMAN BEINGS.** The original investigations of Pirquet and Schick (22) on serum disease showed that second injections of horse serum, made at a considerable interval after the first injection, might give rise to an immediate reaction somewhat like the usual forms of serum disease following the first injection but often of rapid onset and very severe in nature. The analogy to anaphylactic shock in the guinea pig or rabbit is striking and it has usually been considered that the fundamental process of sensitization by the artificial injection of foreign proteins is the same in the human being as in animals. This supposition has, however, recently been called in question by Coca (23), who upholds the view that serum disease is only one form of the so-called "hypersensitiveness," and is dependent upon a

state of reactivity of the tissues of the individual which endows them with a special hypersensitiveness toward the proteins of horse serum, a condition that he terms "allergy" and that he classifies with "drug allergy," "food allergy" and "pollen allergy." All of these "allergies" he believes are fundamentally different from experimental anaphylaxis in the animal inasmuch as he considers that the reactions take place independently of a specific antibody antigen reaction and are inheritable. Most of the arguments which he elaborates against the original conception of Pirquet and Schick, that serum disease is produced by a toxic substance that results from the interaction of newly formed antibody with persisting antigen, are based on such factors as the following: *a*, The irregularities in the length of incubation period; *b*, the conflicting observations upon the results following second injections, the occurrence of immediate reactions, and the difficulties of desensitizing the patient who is "allergic" to horse serum; *c*, the irregularities in the occurrence and relationship of such circulating antibodies as precipitin and anaphylactic antibody to the serum sickness, together with the relationship which the appearance of these antibodies bear to the onset, duration and termination of serum disease; and finally *d*, what he considers to be a remarkable similarity between the phenomena of "drug allergy" and "serum allergy." Before it is possible to discuss intelligently the mechanism of desensitization in the human being, it is important to see in what manner and how far analogies can be drawn between the anaphylactic states in animals and artificial sensitization in human beings.

In spite of the objections which Coca has raised to the view that an antibody antigen reaction is responsible for serum disease and the hypersensitiveness, that is known to develop in the human being after the artificial injection of foreign proteins, the evidence which he brings forward is scarcely sufficient to warrant, at the present time, the separation of serum disease and artificial sensitization in the human being from anaphylaxis as it occurs in animals. When the facts are reviewed there is indeed much to uphold the idea that the two processes are fundamentally the same.

It has long been recognized that different species vary in the readiness with which they may be sensitized to foreign proteins. Guinea pigs may be sensitized with extremely small doses of proteins, dogs and cats are less readily sensitized, rabbits are much more difficult to sensitize, monkeys are highly refractory (24), while rats seem to be entirely refractory (25), (26). Observations upon the effect of injections of horse

serum in man indicate that sensitization can only be accomplished with considerable difficulty, when compared with such animals as the guinea pig. According to collected statistics (27), (28), (29), it requires considerable quantities of horse serum to sensitize man and the incidence of serum disease as well as the subsequent sensitization varies with the dose of serum administered and increases in proportion to the amount. After the injection of 10 cc. of horse serum, only about 10 per cent of patients present symptoms of serum disease, whereas injections of 90 to 100 cc. of horse serum result in serum disease of varying degrees of severity in about 90 per cent of the cases. The variations in the incidence and incubation period of serum disease probably depend upon several factors, but one factor, which is evidently important, is the amount of serum that is administered.

Another factor which has been emphasized by Mackenzie and Leake (30) is the individual variation that is much more striking in the human race than in most species of animals. The observations of Coca, Deibert and Menger (31) upon the incidence of serum disease in the North American Indian demonstrates clearly that members of this race are much less likely to have serum disease, after the administration of 100 cc. of horse serum intravenously, than members of the white race. They give the percentage incidence as 46 per cent in 26 Indians as against 92.4 per cent in 52 whites.

When compared with the results obtained in many animals, single or even multiple doses of horse serum render human beings relatively slightly sensitive to subsequent injections of horse serum; but the observations of Pirquet and Schick, Goodall (32) and Klinenko (33) show that serum disease occurs with greater frequency after second injections of small doses of horse serum than after the first dose. Moreover, when the second injection is made after a considerable time interval, the reaction to the serum is likely to appear within a few minutes to 24 hours following the injection and is frequently violent and of short duration. Goodall states that in 1260 cases receiving a first injection of diphtheria antitoxin 464 or 36.8 per cent developed serum disease; whereas in 203 cases, receiving a second injection from 2 weeks to 13 weeks after the first, 129 or 63.5 per cent reacted. Moreover, the incubation period following the first injection varied from 6 to 15 days in 90 per cent of the cases, whereas in the reinjected cases the serum disease made its appearance within 48 hours in 50 per cent. Furthermore, the first dose of serum may sensitize without in itself producing the visible reaction—namely, serum disease. From the

statistics published by Goodall it seems probable that individuals suffering from an attack of serum disease caused by the first injection are especially prone to react to the second injection. Of 188 patients, half of whom had not had serum disease and half of whom had had serum disease following the first injection of diphtheria antitoxin, 20.4 per cent of the former reacted to a second injection, and 70.77 per cent of the latter. Though all the collected statistics do not agree in detail with those of Goodall, still those of Klimenko and others show a much higher proportion of reactions following the second dose of serum than occur after the first; while the skin reaction, caused by the intracutaneous injection of horse serum, that may be obtained shortly after the occurrence of serum disease, and, which may appear even after a dose of horse serum which has not called forth a generalized reaction, is known to persist for many years. Employed first by Moss (34) as a test to detect sensitization, the skin reaction has been used extensively to determine the presence and degree of sensitization of patients who, for therapeutic purposes, require an administration of antisera months or years after a previous injection. All of the observations point to the fact that the primary injection of horse serum may in itself alter the cells of the body in such a manner that they respond to a second injection as do sensitized animals, with greater rapidity and in a more explosive manner. Inasmuch as serum disease, which may be looked upon as the evidence of a primary reaction, shows considerable irregularity in its occurrence, it is not surprising that the reactions appearing after the second injection are also somewhat irregular in their occurrence, in their severity, in their incubation period and in their duration. Much depends upon the dosage, the method of administration and the interval of time that elapses between the first and second doses. These factors are equally important in determining the effect which is obtained with the second dose of horse serum in sensitized animals.<sup>1</sup>

<sup>1</sup> It is possible, as Taniguchi (*Journ. Path. and Bacteriol.*, 1922, xxv, 77) has recently suggested, that some of the immediate reactions which have been observed in human beings, with the first injection of horse serum, may be dependent upon the presence of heterophile antibodies of Forssman (*Biochem. Zeitschr.*, 1911, xxxvii, 78; Forssman and Hintze, *Biochem. Zeitschr.*, 1912, xlv, 336). Taniguchi points out that heterophile antibody is frequently abundant in human serum, and that horse serum often contains heterophile antigen. The introduction, therefore, of horse serum into the circulation might afford an opportunity for the union of the heterophile antigen injected, with the heterophile antibody in human serum, and give rise to a reaction which is indistinguishable from anaphylactic shock.

Coca has questioned the correctness of the conclusions which have been based on the demonstration of antibodies in the serum and which have been taken by some to indicate that the fundamental principles of sensitization are the same in man and animals. It has been repeatedly demonstrated that man is capable of producing such antibodies toward horse serum as precipitin and anaphylactic antibody, but it is difficult to state that the appearance of these antibodies in the blood serum bears a direct relationship to the onset, the duration or the subsidence of serum disease. Hamburger and Moro (35) believed originally that there was a reaction between precipitin and precipitinogen in the circulation, and that this was the actual cause of serum disease. There seems, however, to be no foundation for this view. Several observers, finding considerable irregularities between the occurrence of serum disease and the appearance of precipitins in the circulation, have concluded that the two processes bear no especial relationship to each other (36), (37), (38); while others (Weil) have thought there was a relationship and, finding precipitin in high concentration in the serum toward the end of serum disease, have advanced the idea that the production of these antibodies in excess is associated with the termination of serum disease, and may be regarded as an effort on the part of the body to dispense with the antigen (39), (40). Some recent experiments of Mackenzie and Fruhbauer (40) have cast doubt upon this latter idea for they have observed that repeated injections of large amounts of antihorse rabbit serum into the veins of rabbits that have been previously treated with horse serum have no effect upon the rapidity of disappearance of horse serum from the circulation of the rabbit. One possible error in drawing conclusions from observations upon precipitin formation, lies in the fact that precipitins and anaphylactic antibody have often been considered as identical. It is not, however, justifiable to apply this conclusion universally, for it has been shown that though the white rat forms specific precipitins for horse serum this animal cannot be sensitized to this protein.

One may conclude, however, that the human being is capable of forming specific antibodies to horse serum such as precipitins and anaphylactic antibody; and that the mechanism of the production of these antibodies conforms to the same general laws as it does in such animals as the rabbit, for though the time of their appearance in the circulation after the first dose of horse serum may differ in the two species, this in both species may be greatly shortened after the second injection of antigen (41).

It is therefore impossible to lose sight of the fact that the human being is capable of reacting to horse serum by the production of specific antibodies toward this foreign protein, and that following injections of horse serum the cells acquire the property of reacting more promptly and with greater vigor to second injections; and it is difficult to escape the original view of Pirquet, which most authors, including Dale and Doerr in their most recent reviews uphold, that serum disease itself is dependent upon an antigen-antibody reaction, and that the subsequent sensitization is due to the presence of antibodies in the tissue cells.

If the conclusion is correct that the injection of horse serum in man produces a state of sensitization which is comparable to that in the guinea pig or other animals, and that is dependent upon the formation in the cells of the individual of specific antibodies toward the protein injected, it should be possible to reproduce in man, as is possible in specifically sensitized animals, the states of specific desensitization and of immunity. To this end the method devised by Besredka has been repeatedly employed with varying degrees of success. In dealing with the sensitized patient it is impossible to determine the minimum dose of serum which will cause symptoms when administered either subcutaneously or intravenously; and it is, therefore, impossible to gauge the doses with any approximation to accuracy. Inasmuch as the second injection is rarely dangerous, unless the serum is given in large amounts intravenously or intraspinally, it is not often that attempts are made to desensitize the patient with accuracy or care. There is considerable evidence to show that the experiments of Otto and Hoefer (42) are inapplicable, and that single doses of horse serum will not desensitize highly sensitized individuals against intravenous or intraspinal injections of horse serum (43), (44), (45), (46). Instances are on record, however (47), in which patients previously injected with horse serum, who were found to be so highly sensitive to this protein that the injection subcutaneously of such small amounts of horse serum as 0.4 cc. called forth the immediate generalized symptoms, could be rendered insensitive to an injection of 8.0 cc. of horse serum intravenously, by giving first 0.025 cc. of horse serum subcutaneously and repeating in increasing amounts the dose of serum; first subcutaneously, and then intravenously. During this process of desensitization mild generalized reactions were observed after 3 of 15 injections. Though few cases that have been carefully studied are on record, it appears to be possible to carry out specific desensitization of man in much the same manner, though not as effectively, as in animals. It is obvious that

absolute desensitization is difficult to accomplish, and that during the process of desensitization, general reactions may follow increases in the dose, especially when this is given intravenously. But the same phenomenon is observed in the desensitization of specifically sensitized guinea pigs and has been commented upon particularly by Weil. There does not appear to be any reason, therefore, to consider that specific desensitization under these conditions is essentially different in man and animals.

There seems to be little accurate information concerning the possible immune state in man. Repeated primary injections of horse serum, given at short intervals, have no effect upon the incidence or the incubation period of serum sickness itself, for it cannot be prevented by this method. There does, however, seem to be a period following serum disease, in which the patient shows considerable resistance to second injections of the same serum. Goodall found that of 45 patients, reinjected within 5 weeks of the first injection, only 37.7 per cent showed serum disease while of 47 reinjected after the 27th week 76.5 per cent showed serum disease. It does seem to be possible to administer horse serum in large doses intravenously, ten days after an attack of serum disease, without causing more than a mild transitory reaction (48). It is further known that precipitins and indeed anaphylactic antibody (49)(50) may persist in the circulation for many days, after an attack of serum disease, and though experience upon the reinjection of individuals with serum at intervals of a few weeks is very limited, there are observations that suggest that a period of considerable resistance may follow an attack of serum disease. These few available data suggest that this period of comparative insensitiveness corresponds to the period of immunity in animals following repeated injections of protein at short intervals; and that this immunity may be susceptible to the same explanation; namely, the presence of an excess of circulating antibodies that prevents the union of antigen with the cells of the body.

**DESENSITIZATION OF THE NATURALLY HYPERSENSITIVE STATE IN MAN.** Since the report in 1909 of Gillette (51) who collected accounts of 30 cases of sudden collapse or death following the first injection of diphtheria antitoxin, there has been considerable and increasing interest in the forms of hypersensitiveness, already alluded to, which occur in hay fever patients, in asthma, in certain acute gastro-intestinal disturbances, in eczema, urticaria, angioneurotic edema and some other forms of cutaneous eruptions. The work of Cooke and Vanderveer (52), Schloss (53), Talbot (54), Blackfan (55), Goodale (56), Walker (57), Rackemann (58), Koessler (59), Scheppergrell (60), Hurst (61), and Free-

man (62), has established in great numbers of cases a direct etiological relationship between a hypersensitiveness toward one or several of a great variety of substances and the symptoms manifested by many patients suffering from these diseases.

From a broad point of view, it seems apparent that hypersensitiveness shown by different people to pollens, animal emanations, foods, drugs, and possibly bacteria, is dependent upon a common pathological state. The symptoms, it is true, arising from this state of hypersensitiveness may vary in different patients, for in one person they may indicate involvement of the respiratory tract, in another, of the gastro-intestinal tract, or in a third, some affection of the skin; but on the other hand, in many reported instances of hay fever, or asthma, there have appeared simultaneously or alternately in the same individual gastro-intestinal disturbances and skin eruptions, and so far no arguments have been adduced to lead one to believe that the fundamental alterations in the tissues of the patient who suffers from hay fever due to ragweed is essentially different from those of a similarly hypersensitive patient who suffers from urticaria or gastro-intestinal disturbances from eating eggs. As a matter of fact the ingestion of a single food, such as eggs, may cause in the same person or in different persons, such varied symptoms as urticaria, eczema, asthma and gastro-intestinal disturbances. The reason why a single substance, such as egg, should cause asthma in one person, urticaria in another and gastro-intestinal disturbances in a third, has not been satisfactorily explained, but the experiments of Auer (63) and an observation of McBride and Schorer (64) suggest that non-specific local irritation may render one organ or tissue of the body more susceptible than another to the reactions brought about in a hypersensitive individual by contact with the specific material to which he is sensitive. Auer found that the tissues of the sensitized rabbit's ear, irritated by the application of xylol, undergo edema and necrosis when the antigen is reinjected intravenously, in doses too small to cause symptoms of anaphylactic shock, while McBride and Schorer record the history of a man hypersensitive to eggs in whom, after eating small amounts of egg white, urticaria appeared only when the skin was irritated mechanically.

A comparison of these types of hypersensitiveness seen in hay fever, asthma, etc., which have often been termed "natural," since their origin is very obscure, both with the artificial hypersensitiveness produced in human beings by the injection of such foreign proteins as horse serum, as well as with the anaphylactic state produced experi-

mentally in animals, discloses, on the one hand, important analogies, and, on the other, brings out differences that have seemed to some investigators to be almost of fundamental importance.

Coca again upholds the view that the so-called allergies are fundamentally different in their nature from the anaphylactic state in animals. It is true that there are at first sight striking differences between the anaphylactic animal and the hypersensitive individual. In the first place the etiology of these natural forms of hypersensitiveness is still obscure, for though it has been shown by Schloss and Worthen (65), Schloss and Anderson (66) and Grulee and Bonar (67) that a fair proportion of infants absorb egg albumen from the gastro-intestinal tract and excrete this protein unchanged in the urine, and by Shannon (68) that suckling infants may acquire through their mother's milk proteins which form part of their mother's diet, it has not been definitely proven that the parenteral introduction of such foreign proteins may always produce sensitiveness in infants. Indeed Stuart (69) has been unable to confirm the work of Shannon by careful experimentation and has failed to demonstrate egg protein in mother's milk.

There must be some other factor which determines the extreme hypersensitiveness of patients subject to hay fever, asthma, urticaria and similar conditions, for it is known that man is less readily sensitized by artificial methods than many of the lower animals, and it does not seem likely that during the absorption of small amounts of protein through the mucous membranes of the gastro-intestinal tract, or of the respiratory tract or even through the skin could alone produce the exquisite hypersensitiveness which is often observed in the human being, and which may result in the appearance of violent symptoms after the subcutaneous injection of 0.000,001 cc. of antigen.

One other factor that must be considered is the hereditary tendency that many families show to idiosyncrasies of various types. The familiar tendency toward asthma has been frequently alluded to by most writers on this subject. It has now been established that hypersensitiveness to proteins may occur very definitely in families, and Cooke and Vanderveer have brought out the fact that hay fever affects members of families in a proportion which closely approximates the theoretical figures of the Mendelian Law. They suggest that such idiosyncrasies may be inherited as a dominant characteristic. In their study of 504 cases of sensitization, principally to pollens, in which a satisfactory history could be obtained, 48.4 per cent showed some form of sensitization in the direct or collateral antecedents. The history was positive for one

side of the family alone in 205 cases and for both sides in 39 cases. Rackemann (70) found, in a study of 150 cases of asthma, that a history of asthma, hay fever, or food poisoning in the immediate family could be obtained in 58.7 per cent of the cases that showed hypersensitiveness to foreign proteins, but in only 10.5 per cent of the instances that were not demonstrably hypersensitive. Walker has likewise emphasized the familial tendency to hypersensitiveness in asthmatics. Striking examples of familial idiosyncrasies have been reported by Talbot, while Laroche, Richet and St. Girons (71) have reported a family in which an idiosyncrasy to eggs ran through four generations and occurred usually in the male members. Adkinson's (72) very careful analysis of the factors of inheritance in 400 cases of asthma, studied by Walker, brings out many important points. It was found that 48 per cent of the cases gave a history of the occurrence of asthma among other members of the family. In the group of 191 of these patients that were found sensitive to protein, 52 per cent gave a history of asthma in the family, while in the non-sensitive group 41 per cent knew of the occurrence of asthma or hay fever in the family. It was further found that many of the asthmatic families contained both sensitive and non-sensitive individuals, though sometimes sensitization ran definitely in families. In the instances of familial sensitization, the sensitization was not identical as regards the specific proteins or the clinical symptoms in different members of the same family. Adkinson concludes that the tendency to asthma acts as a true inherited trait, and may be transmitted as a dominant or as a recessive characteristic. In the extreme dominant, where both parents have asthma or hay fever, all the children tend to develop the condition, whereas in the extreme recessive, when both parents are normal but one is simplex, half the children would be simplex and bear the asthmatic characteristic recessive in their germ plasm. The results of all these studies and observations seem to establish the fact with considerable assurance that a condition of the tissues, at least, may be inherited that renders the individual highly prone to the development of hypersensitiveness. The idiosyncrasies themselves may differ in the different members of the same family and may assume quite different forms of expression. Occasionally, all members of the family may have hay fever, but in some it may be caused by ragweed and in others by timothy. On the other hand, one member may have hay fever, another horse asthma, and a third egg eczema and urticaria.

The age incidence of the condition of natural hypersensitiveness has been a subject of considerable study. Most observers have pointed

out the fact that it is during the first few decades of life that the manifestations of the spontaneous hypersensitiveness usually occur, and that it is likewise during this period that the tissues react most violently to the application of the protein or foreign substance to which they may be hypersensitive. Walker was rarely able to detect specific hypersensitiveness in asthmatics over the age of 50 and finds that the detection of hypersensitiveness is most readily accomplished in the first and second decade. Rackemann (73) emphasizes the same fact and Latham and Coke (74) state that the onset of symptoms of sensitization in the 270 cases of asthma that they studied was rarely after the age of 30. Coca (75) has compared the age incidence of serum disease and of dermatitis venanata with that of the natural allergies. From observations and theoretical calculations he concludes that 11.6 per cent of the potentially susceptible individuals present symptoms by the fifth year; 27.8 per cent present symptoms by the tenth year; 43 per cent by the fifteenth year, and that by the forty-fifth year practically 100 per cent of the hypersensitive individuals have manifested this state by some symptom. On the other hand the age incidence of dermatitis venanata increases greatly from childhood to adult life at which time it reaches a high figure affecting 90 per cent. The susceptibility to serum disease is quite different from either of these, for according to Coca's statistics all ages are equally affected and the incidence shows practically no variation in the different age groups.

Another characteristic of the individual who shows these idiosyncrasies is that his tissues are likely to react to more than one substance, and as a rule about one-half of the patients show what has been termed multiple sensitization. (Cooke and Vanderveer, Walker, Caulfield, Longcope, Rackemann.) The variety of material which calls forth these reactions and which is known to produce the symptoms in many of these hypersensitive individuals, is very great and is not confined to the proteins themselves or to those substances producing antibodies when injected into animals. Not only are reactions obtained to protein containing substances but symptoms may also be produced and skin reactions obtained from the use of a variety of drugs and chemicals. Since these latter substances contain no protein, as far as one can determine, it is difficult to explain their action upon the same hypothesis as that used to elucidate the anaphylactic reaction in animals.

Though multiple sensitizations are common in the idiosyncrasies and occur in 50 per cent, the groups of substances to which the patients are sensitive are not always of one type, and though certain individuals

may react to several varieties of plant pollen (52) and others to the protein of eggs or to the extract of vegetables only, this is by no means uniform, and frequently individuals are encountered who may react not only to pollens but to the extract of animal dust and to egg albumen (54), (57), (53), (100), (58). Also, in spite of these multiple reactions, there seems often to be a specific selection among different proteins which go to make up a complex substance such as egg white, cereal seeds or animal hairs. The patient may show a typical reaction with ovomucin, but none to conalbumin (Schloss), a typical reaction to wheat proteose, but none to wheat gliadin or leucosin; to horse dander, but not to horse serum; or to the peptone of dog hair, but not to the alkali metaprotein of dog hair, or vice versa (Walker). It is difficult therefore to understand this multiple sensitization on the basis of a group reaction or upon the basis of non-specific sensitization.

It is interesting, too, to note that the presence of circulating antibodies in these naturally hypersensitive individuals is extremely rare. Isolated reports are on record of specific precipitation, of complement fixing antibodies and of passive transfer of sensitiveness to guinea pigs with the serum of individuals who show idiosyncrasy to such varied antigens as pollen, egg white and the extract of horse dander (76), (77), (59), (78), (79); but many observers have failed consistently to obtain such results (80), (81). One instance has been reported by Ramirez (82) of possible transfer of hypersensitiveness to horse dander by transfusion of blood from one human being to another, and if this, by accident, should be repeated, it would have important bearing on this entire subject.

From the data that have accumulated one must conclude that natural idiosyncrasies for allergies which occur toward various proteins and even non-protein substances, such as drugs, differ in many respects from anaphylaxis in animals and indeed from the artificial sensitization in man, the prototype of which is serum disease, so that one cannot at the present time be sure that this group of individuals who so frequently suffer from hay fever, asthma, eczema and urticaria ought to be considered in the same category as animals sensitized to foreign proteins. On the other hand, there are many similarities as Dale (83) has recently pointed out, and if one assumed that the cells of these individuals for some reason attained a very high degree of sensitiveness sometimes to one, sometimes to several antigens, the fundamental condition existing in these hypersensitive individuals would be analogous or almost exactly the same as that which is observed in the sensitized guinea pig.

Like the specifically sensitized animal, these individuals may also be desensitized. The early attempts of Noon (84), Freeman (85) and Clowes (86) to render patients with hay fever refractory to inhalation of the specific pollen to which they were sensitive was soon repeated by Goodale (87), Koessler and Cooke (88), and have been greatly extended and amplified especially by Cooke and Vanderveer, Walker, Rackemann and others. These workers have established the fact that a certain proportion of patients may be relieved of their symptoms partially or entirely by injecting subcutaneously minute quantities of extracts of the pollens, to which they are hypersensitive, in increasing doses at 5 to 7 day intervals. To be effective, the injections must be started several months before contact is likely to occur with the specific pollen, and must be continued for weeks or months. Vanderveer (89), reporting on 2000 cases of hay fever, states that 25 per cent of cases are entirely relieved of symptoms, 50 per cent of patients are made comfortable, 15 per cent are slightly relieved and 10 per cent are not helped. Somewhat similar results have been obtained by Walker (90) and by Rackemann (91). Though the proportion of cases of asthma sensitive to animal dust, pollens and foodstuffs that may be relieved by this method of treatment is somewhat smaller, still a certain number show improvement of symptoms following this method of treatment (92). Not only may this hypersensitiveness be reduced by subcutaneous injections, but it may also be brought about by local application of the specific substance to mucous surfaces; for Schloss (93) showed that a child, who was highly sensitive to the ingestion of egg white, could be rendered refractory by feeding him small quantities of ovomucoid in capsules. The dose was gradually increased so that at the end of 2½ months the child was able to eat eggs without symptoms. This observation, confirmed by Talbot (94), has been employed repeatedly with success not only with eggs but with milk and other food substances (95), (96), (97). It is not only in the cases of alimentary sensitiveness that application to the mucous surface of the specific substance causing the reaction brings about desensitization, for it seems probable that certain forms of hypersensitiveness of the respiratory tract may be relieved by the direct application of the specific antigen to the nasal mucosa. The experiments of Sewall (98) showed that the local application of horse serum to the nasal mucosa of guinea pigs may sensitize the guinea pig both locally and generally, and that repeated nasal installations of small amounts of horse serum would sometimes result in desensitization of the nasal mucosa without impairment of the

general sensitiveness. Similar observations have been made by Ulrich who employed in his experiments pollen extract and pollen dust. MacKenzie and Baldwin (99) have employed a somewhat similar procedure in an attempt to desensitize patients with hay fever by local application to the nasal mucosa of pollen extract. They find that the reactivity of the nasal mucosa may be markedly diminished by spraying the nose and throat with the specific pollen antigen, and that the results compare favorably with those obtained by giving repeated subcutaneous injections of the pollen antigen. Caulfield (100) is inclined to uphold this view. The most satisfactory evidence that the repeated local application to a tissue of an antigen results in diminished reactivity of this tissue is to be found in some experiments of MacKenzie and Baldwin (101). They were able to render a local area of the skin, which was hypersensitive to different antigens, insensitive to these antigens by applying, at short intervals, solutions of the antigen to a given area; while the skin only a short distance from this selected spot retained its original hypersensitiveness. This local exhaustion of the reaction was found by them to be specific but of short duration, lasting only 24 to 48 hours. Cooke (102) in repeating these experiments points out that the local exhaustion of the reaction is only relative, and that when the area no longer responds to a high dilution of antigen it may still react to a stronger dilution. In his experiments the exhaustion was not absolutely specific.

The materials, such as foods, pollens and animal emanations to which patients may be hypersensitive usually contain protein, and can therefore be considered as true antigen; but hypersensitiveness is often observed to chemicals and drugs that obviously are not protein in nature, and, therefore, cannot be considered antigens in the ordinary sense of the word. In spite of this fact patients hypersensitive to drugs may respond with cutaneous reactions and general immediate symptoms from the local application or ingestion of drugs as they do to pure proteins (103). Such patients are likewise susceptible to desensitization and may be made refractory to quinine (104), (105), (106), to antipyrine (107), as well as to the other chemical substances by repeated doses of minute quantities of the specific drug to which they are hypersensitive.

There is abundance of proof, therefore, to show that individuals who are spontaneously hypersensitive to such varied substances as pollens, animal emanations, foods and chemicals such as alkaloids and crystalloids may, by various methods of application of the specific substance,

be rendered partially or almost completely insensitive to fairly large doses of the material which, before treatment, would have caused definite or violent symptoms. The relative degree to which this state of desensitization may be carried is illustrated by records of a case published by Alexander (108). This patient, an asthmatic, suffering with pneumonia was found to be spontaneously hypersensitive to horse serum and to extracts of horse hair. By fractional dosage such large quantities of antipneumococcus horse serum as 65 cc. could be administered intravenously without producing more than mild symptoms of asthma. Although this patient was rendered refractory to large doses of horse serum, the records show that there was not complete abolition of the hypersensitive state, and Cooke (109) from his wide experience concludes that it is practically impossible to render a patient who is spontaneously hypersensitive to a given substance completely refractory to very large doses of this material. To uphold this view is the fact that patients undergoing desensitization may frequently react with considerable violence to injections of the antigen when the doses are increased too rapidly. Indeed all who have employed this method have warned against these reactions (110), and Vanderveer states that in 1 to 2 per cent of his cases severe reactions occurred during the process of desensitization.

The desensitization or hyposensitiveness is only of temporary duration. In the local skin desensitization obtained by Mackenzie and Baldwin it lasted but a few days. In the case of antipyrine sensitiveness reported by Widai and Vallery-Radot, the sensitiveness was greatly reduced by administering repeatedly small doses of the drug, but recurred 43 days after the last desensitizing dose. It is well known that the relief which hay fever patients obtain, after a series of inoculations, rarely lasts for more than one season (Cooke, Vanderveer, Walker). It is usually easier to accomplish desensitization the second year than it is the first.

Since the mechanism of the sensitization or the allergy in the spontaneously hypersensitive individuals is not clearly understood, it has been found difficult to explain the process of desensitization. It seems clear that the desensitization may be highly specialized and that desensitization may be as specific as it is in the anaphylactic guinea pig.

The state of desensitization is not known to be accompanied by the presence of specific antibodies in the circulation (Cooke, Floyd, Coca and Rackemann). But this problem has been somewhat opened again by the observations of Wood (111), who found that during desen-

sitization both of dogs and of patients hypersensitive to the choroidal pigment of the eye, specific complement fixing antibodies appear in the circulation. The sensitization to pigment which occurs in these patients may be more nearly like the artificial sensitization to injections of horse serum than it is to the spontaneous hypersensitiveness of asthma and hay fever, and it would therefore be improper to draw conclusions from Wood's experiments that could be applied to the latter group of cases.

Coca, Floyd and Cooke (112) drew the conclusion from their experiments, devised to produce anaphylaxis in guinea pigs with extracts of ragweed pollens and of horse epithelium, that the phenomenon of desensitization in the hay fever patient is dependent upon an antigen-antibody reaction and that it is essentially the same as the desensitization of the guinea pig anaphylactic to horse serum. Later, however, Coca dismissed this view as lacking in proof. Though proof of an antigen-antibody reaction is difficult to establish, there are many analogies between the phenomenon of desensitization in the specifically sensitized guinea pig and the naturally sensitive hay fever or asthmatic patient, and it does not seem unreasonable to suppose, as some observers have, that during the period of desensitization a reaction takes place between the antigen and some form of antibody in the cell, during which process the antibody in the cell is reduced in amount or partially exhausted. As the exhaustion becomes greater, larger and larger amounts of antibody are required to call forth a reaction. When, by cessation of the injections, antigen is no longer furnished, the reacting substance in the cells returns, as it does in the anaphylactic guinea pig, and hypersensitiveness is reestablished.

**NON-SPECIFIC DESENSITIZATION.** There are other theories, however, that have been presented more recently and which are based upon the conception that desensitization may be brought about by non-specific as well as by specific measures. It was originally shown by Besredka that the symptoms of anaphylactic shock in the guinea pig might be suppressed or reduced by narcotics. Ether, chloral, chloroform, alcohol and ethyl chloride were all more or less effective in diminishing the symptoms of shock or preventing the death of the animal; and he supposed the result was dependent upon a lowering of the sensibility of the animal to the effects of the anaphylactic shock. Friedberger and Hartoch (113) demonstrated that a dose of 0.1 to 0.3 gram of sodium chloride, given immediately before the shocking dose of protein, will protect sensitized guinea pigs against a full lethal dose of the specific protein. C. Richet, Brodin and F. Saint-Girons (114), from their experi-

ments upon the action of sodium chloride in suppressing anaphylactic shock in dogs, conclude that it is due to a saturation of the cells of the body by sodium chloride which makes them impermeable to the hypothetical poison produced during anaphylactic shock. More direct evidence to explain the protective action of sodium chloride has been brought forward by Dale and confirmed through some experiments with proteotoxins by Zinsser, Lieb and Dwyer (115). These experiments showed that the addition of sodium chloride to the solution in which the uterus from a sensitized guinea pig is suspended, rendered the muscle refractory, and prevented the contractions that usually occur upon the addition of the specific protein to which the animal is sensitized. It seems probable that the inhibition of the reaction under these circumstances is due to the lessened irritability of the muscle cells.

It has further been observed that many chemicals when injected immediately before the intoxicating dose of specific protein will abolish or reduce the symptoms of anaphylactic shock in guinea pigs and dogs. Biedl and Kraus (116) observed such an effect with barium chloride, Lumière and Chevrotier (117) with sodium sulphate, Brodin and Huchet (118) with a combination of formaldehyde and hydrosulphite of sodium, Kopaczewski (119) with cocaine, saponin, soaps, biliary salts, alkaline carbonates, saccharose and glycerine; Van Geertryden, Bernard and Zunz (120) with hirudin, and Archard and Flandin (121) and Duprey (122) with injections of lecithin.

A possible explanation for the protective action of some of these alkaline salts, which has also been observed by Sicard and Paraf (123) and which, in the form of intravenous injections of sodium carbonate, has been employed by them as well as by Widal, Abrami and Brissaud in the treatment of serum disease, may be found in some experiments by Eggstein (124), who discovered, that, during anaphylaxis in dogs, there occurs a reduction in the carbon dioxide capacity of the serum which appears at the first symptom and falls progressively during shock, often reaching as low a figure as 25 volumes per cent. If recovery occurs the carbon dioxide capacity of the serum returns to normal within 6 hours. Though German (125) was not able to confirm these observations during anaphylactic shock in rabbits, Hirsch and Williams (126) have noted a considerable decrease in the  $\text{CO}_2$  combining power of the plasma during anaphylactic shock; and, by the gas chain method, have found a change in the pH of the blood to the acid side which was notable in some instances and showed a reduction that was as great as from 7.8 to 6.88. These observations, though somewhat conflicting, would indi-

cate that a pronounced acidemia develops during anaphylactic shock, and that the administration of alkaline salts at this time is followed by some amelioration of the symptoms. It seems improbable, however, that the acidosis can be looked upon as anything more than a symptom, and a sequel to more profound alterations that take place in the cells and tissues of the body. Other chemical changes may occur in the serum at the same time for Stern and Reiss (127) state that the blood lipoids and neutral fats of the serum decrease during anaphylactic shock in dogs.

It has been generally assumed by many that the action of most of the chemicals, which, so to speak, are non-specific in their action and purely transitory in their effect, is directed toward the relief of symptoms or toward rendering the tissues of the body more resistant to the effects of the hypothetical toxin produced. They protect the animal or individual in much the same way as do adrenalin and atropin. More recently, Kopaczewski (128) has advanced the view, that many of these chemicals have, in common, an effect which, he believes, is of fundamental importance in preventing anaphylactic shock. In studying the production of anaphylatoxin, Kopaczewski (129) found that the toxin could be produced from blood serum by the rapid contact with gels at 0°C. He concluded that the formation of this substance could not be dependent upon ferment action. Observing that a conglomeration of particles occurred in the serum during the formation of anaphylatoxin, he conceived the idea that the transformation of a bland serum into a toxic serum was caused by this flocculation. The flocculation of colloids is attended by certain physical changes in the serum which he considers to be of much importance. These changes consist in a lowering of the surface tension and increase in viscosity of the blood. Upon the basis of these experiments he has constructed an hypothesis to explain the production of anaphylatoxin shock and anaphylactic shock, as well as of desensitization. In this, he assumes that, during shock, there is a rupture of the colloidal equilibrium that is expressed by flocculation of particles. He finds that chemicals, which prevent the lowering of surface tension of serum, inhibit the formation of anaphylatoxin *in vitro*, and possess some power to inhibit the symptoms of anaphylatoxin shock or anaphylactic shock *in vivo*. Though saponin and the soaps are particularly effective in this respect, he believes that most of the chemicals, that exhibit an ameliorative action in anaphylactic shock, owe their power to this particular property.

Since the hypothesis rests upon the detection of certain physical changes in the serum, rendered anaphylatoxic *in vitro*, it is important that these observations should be repeated. Zunz and LaBarre (130) state that they have observed the same changes in the surface tension and viscosity of the serum during anaphylatoxin formation; but Dale and Kelloway (131) have been unable to detect, by accurate methods, any change in the surface tension or viscosity of blood serum rendered toxic by incubation with starch.

Though questioning the details of some of Kopaczewski's work, many of the recent French writers are inclined to conceive of a physical change as the essential feature of anaphylactic shock. Widal, Abrami and Vallery-Radot (132) have adopted the view that the symptoms do not depend upon the production of a poison but arise from physico-chemical changes in the equilibrium of the body fluids and cells. They believe that in anaphylatoxin shock the rupture of the colloidal equilibrium or "colloidoclasie," as they term it, takes place in the blood serum, while in specific anaphylactic shock it proceeds within the body cells. They take exception to many of Kopaczewski's ideas but agree in considering that the process is not fundamentally specific in nature, and advance the view that any substance that brings about the rupture of colloidal equilibrium, to a slight extent or by slow degrees, will prevent the explosive rupture which induces the condition of acute anaphylaxis. They do not seem to make any differentiation between the specific desensitization, as developed by Besredka, and the non-specific inhibition of anaphylactic shock, and conclude that the mechanism in both is the same.

A practical application of this hypothesis is to be found in the work of Pagniez and Vallery-Radot (133) who advocate the administration of peptone in capsules by mouth to prevent the urticaria or other symptoms caused by eating foods to which the individual is hypersensitive.

A type of non-specific desensitization brought about by the use of organic compounds was first described by Biedl and Kraus (134) who stated that animals, sensitized to a specific serum, could be desensitized with heterologous substances such as peptones. Besredka, Strobel and Jupille (135) were unable to confirm this particular observation in guinea pigs, and Besredka (136) more recently, in attempting to differentiate anaphylatoxin shock from anaphylactic shock, found that sensitized guinea pigs, rendered immune to the injection of suspensions of agar as described by Bordet (137), showed no reduction in their sensitiveness to the specific protein.

The literature, however, contains many observations that uphold, in a general way, the observation of Beidl and Kraus, and that indicate that the intravenous injection of heterologous proteins in animals, specifically sensitized, diminishes the sensitiveness to injections of the specific antigen. Experiments upon the specific desensitization of animals treated with two or more separate proteins, has brought out the fact that desensitization to one protein may render the animal somewhat less sensitive to the second. Bessau (138) sensitized guinea pigs to two different antigens and after producing shock by the reinjection of one antigen tested the sensitiveness of the guinea pig to reinjection of the second. He found that the sensitiveness of the guinea pig was materially reduced, under these circumstances, to the second antigen, as measured by the controls; and that this partial desensitization increased with the severity of the shock caused by injection of the first antigen. In later experiments (139) he measured more accurately the effects of the shock induced by the first protein in desensitizing towards the second and found that the period of desensitization persisted for 14 days. Friedberger, Szymanowski, Kumagai and Odaiva (140), who studied this problem extensively, concluded that though specific desensitization, under the above conditions, can be carried out to relatively high degrees it is not absolute. Dale and Hartley (155) have observed that the uterus of a guinea pig sensitized to two different antigens reacts, *in vitro*, more violently upon the addition of the first antigen than upon the addition of the second; and though reactions are obtained, which are to a high degree specific, the primary contraction of the uterus does effect to a certain extent the reaction of the uterus to the second antigen, so that in many instances the maximum effect is not obtained. Marsini, (141) using the intestinal strip of the guinea pig to detect desensitization in guinea pigs sensitized to two antigens, came to the conclusion that there was both a specific and an aspecific "antianaphylaxis." Brack (142) has recently reinvestigated this problem. He sensitized guinea pigs with rather large doses of three antigens, sheep serum, horse serum and human serum; and employed the intestinal muscle strip to measure the degree of sensitization. He found that application of one antigen, which produced a maximum contraction of the muscle, rendered the muscle much less sensitive than the control to application of the second antigen; while the muscle which had contracted upon the application of two different antigens responded very feebly to the third. He suggests that the phenomenon may depend somewhat upon a diminished reactivity of the smooth muscle to any form of stimulation.

But this cannot explain the phenomenon entirely, for in other experiments a similar, though not quite so marked, reduction in sensitiveness to application of the second protein could be obtained by repeated additions of small quantities of the first antigen, insufficient to cause contractions, but in aggregate, enough to bring about complete desensitization to the first protein. It seems impossible to avoid the conclusion that in animals sensitized to two proteins, anaphylactic shock to one reduces temporarily the sensitiveness to the second.

The same effect as that noted in experiments upon double sensitization and desensitization may, indeed, be obtained by the injection of large doses of heterologous proteins, for Pfeiffer and Mita (143) were able to protect guinea pigs, sensitized to horse serum, against a lethal dose of this antigen by a previous injection of beef serum. Beef serum seems to be much less likely than horse serum to cause serum disease in human beings. This has been pointed out by Kraus, Cuenca and Sordelli (144) and it has therefore been advocated by Kraus (145) that cattle be used for the preparation of antitoxic sera. Calvary (146) states that some of the effects of specific anaphylactic shock in dogs, such as the increased flow of lymph and the drop in blood pressure, can be inhibited by an injection of beef serum made previous to the injection of specific antigen. Benjamin and Witzinger (147) observed quite definitely the partial inhibition, by injections of heterologous sera, upon anaphylactic shock following the injection of the specific antigen. Brack records similar observations. Karsner and Ecker (148) have recently reviewed the entire subject of non-specific desensitization by the use of heterologous sera upon anaphylactic shock following the injection of the specific antigen. Beef, swine, ox, sheep, rabbit and human sera were chosen as heterologous sera. Since the minimal lethal dose of horse serum was not determined in their experiments, it was difficult or impossible to measure the effectiveness of desensitization by homologous sera, but this was found frequently to be considerable. They noted, however, that, to a certain extent, desensitization could be accomplished by the use of heterologous sera; and that the most effective way of inducing the non-specific effect was through intravenous injection. The heterologous desensitization developed with as great rapidity as the homologous desensitization but was of distinctly shorter duration. Dale (149) has devoted some attention to this problem of non-specific desensitization and accepts its accomplishment as a fact. Kelloway and Cowell (150) have confirmed some of the previous observations upon the desensitizing property of anaphylatoxin for guinea

pigs actively sensitized to a single protein. The anaphylatoxic serum which they employed was prepared from guinea pig serum, and, during the experiments, they noted the interesting fact that the intravenous injection of normal guinea pig serum into actively sensitized guinea pigs, causes a definite protection against the specific antigen which, however, is of short duration. By employing the uterine strip preparation, they could show, that this loss and subsequent return of sensitiveness of the anaphylactic animal, ran parallel to, and could be explained by changes in the sensitiveness of the smooth muscle. They consider that these changes are due to physical alterations in the muscle cells.

Though it seems quite clear from many of the foregoing experiments that the active sensitization to one antigen may be temporarily reduced by the intravenous injection of heterologous proteins, as well as of toxic substances derived from proteins, and, though it is evident that demonstrable intoxication by these heterologous proteins and anaphylatoxins, is not essential for the partial desensitization, the exact explanation of this non-specific effect is still obscure. It is usually spoken of as desensitization, but there is no direct evidence to show that these preliminary injections produce an effect upon the specific antibodies residing in the body cells.

Doerr has pointed out the fact that if complex antigens, such as animal sera, are employed to immunize rabbits for the production of precipitating sera, crossed or group reactions are not uncommonly encountered, especially when the animals are highly immunized. These group reactions are more frequent when the sera of nearly related species are used in the tests. Consequently, when complex antigens are employed for double sensitization and desensitization, it is conceivable that crossed or group reactions may be encountered, and that during the anaphylactic shock, caused by the injection of one antigen, a certain proportion of the antibodies to the second antigen may be neutralized or destroyed. These group reactions are, however, almost entirely eliminated when pure antigens are employed (151). The most suggestive experiments dealing with this point are those of Kellaway and Cowell. They observed that the intravenous injection of normal guinea pig serum into guinea pigs, immunized against horse serum, resulted in an immediate and marked decrease in the circulating antibodies. This resulted in an enhanced sensitiveness to injections of the specific antigen of guinea pigs that were immunized, were known to contain a high titre of circulating antibody, and that could be shown, by controls, to be quite refractory to injections of the specific antigen.

This enhanced sensitiveness occurred during two periods; first, shortly after the intravenous injection of normal guinea pig serum and secondly after the restoration of the sensitiveness of the plain muscle, but before the return to normal of the demonstrable circulating antibody. These experiments are highly suggestive of a direct action of the heterologous serum upon the specific antibody.

There are, however, other factors of importance that must be considered. These have to do with certain antagonistic effects that have been observed between two foreign proteins when they are injected simultaneously or in rapid succession. The early experiments of Benjamin and Witzinger showed, that when two antigens are injected into a normal animal simultaneously or in succession, they may call forth unequal reactions on the part of the body. This is particularly true when one antigen is in excess of the other. They showed in guinea pigs that when an injection of a large dose of horse serum was followed, in 24 hours, by a smaller dose of beef serum, sensitization to the beef serum is inhibited or suppressed. Weil (152) has found that several injections of large amounts of heterologous serum will prevent passive sensitization of guinea pigs to a specific immune serum. He injected normal rabbit serum or sheep serum into normal guinea pigs, and found that, for 24 hours to 14 days following these injections, the guinea pigs could not be passively sensitized to horse serum by the injection of serum from rabbits immunized to horse serum. As an explanation, he suggested that the large amounts of rabbit serum or sheep serum saturated the receptors capable of forming antibody against foreign protein, so that a union, between the horse serum antibody and the cells could not take place. Julian Lewis (153) has shown very clearly how marked may be the inhibiting effect of the injection of large doses of serum from one species, in preventing active sensitization in guinea pigs against small doses of serum from other species. Thus an injection of 2.0 cc. of dog serum inhibited active sensitization of guinea pigs to an injection of 0.3 of horse serum. This inhibitive action may take place when both foreign sera are injected together.

Some recent work of Doerr and Berger (154) goes to show that this effect is not altogether a quantitative one. They have studied the anaphylactic reactions in guinea pigs to four different protein fractions of horse serum, namely Euglobulin, Pseudoglobulin, Albumin C and Albumin D. They worked principally with Euglobulin and Albumin C, and found that guinea pigs could be readily and specifically sensitized to these two fractions, but that for animals sensitized to equal amounts,

the intoxicating dose of Euglobulin was considerably smaller than that for Albumin D. A guinea pig, injected simultaneously with equal quantities of Euglobulin and Albumin D, became equally sensitive to the two proteins; but, when one antigen was in excess of the other at the primary, injection, though both were given simultaneously, very interesting results were obtained. It proved that an excess of Euglobulin inhibited sensitization to Albumin D, but the reverse was not true; and an excess of Albumin D did not prevent sensitization to Euglobulin.

It is possible that such experiments with purified proteins will throw considerable light upon the problem of non-specific desensitization and the inhibition of sensitization. Heretofore most experiments directed toward the elucidation of the problem have been carried out with such complex substances that it is difficult to unravel and explain the results that are sometimes discordant. It seems highly probable, that the cells of the body do not react, with the same degrees of intensity and of rapidity, toward different proteins; and for a proper understanding of this whole question it is essential that further information should be obtained upon these important biological and chemical reactions. Dale and Hartly (155) have shown, that, when guinea pigs are sensitized to serum globulin or to serum albumen, there is a distinct difference in the time interval required for the appearance of sensitiveness of the uterine muscle to these two proteins. The sensitiveness to serum albumen appears several days before that of serum globulin. Doerr and Berger (156) have extended their experiments upon the fractions of horse serum and find that there are distinct biological differences between the activity of these fractions. These consist in differences in the size of the minimal sensitizing dose of the proteins, which was found to descend in the scale from Euglobulin to Albumin D; in differences in the incubation period, which, for a given amount of protein, was found to be shortest for Euglobulin and longest for Albumin D; and in differences in the amounts of protein required to produce anaphylactic shock in actively sensitized guinea pigs. Euglobulin produces shock in the smallest doses; Albumin D in the largest.

It can be seen from this review that many of the details of the mechanism of desensitization of animals or of human beings, actively sensitized by the injection of specific protein, and of animals, passively sensitized to specific proteins, are imperfectly understood; but most of the experimental evidence points to the fact that, during the process of specific desensitization, there is a neutralization or destruction of antibodies situated in the cells of the animal. To accomplish complete

destruction or neutralization of these antibodies is extremely difficult in highly sensitized animals or human subjects.

Suppression of anaphylactic shock, which has an entirely different significance from desensitization, may be brought about by various methods, depending in some instances, upon the reduction of reactivity of the tissues of animals to the intoxicating factor of the shock itself; and accomplished in other instances by placing a barrier between the cells of the body and the specific antigen which is injected.

It is possible that the so-called non-specific desensitization, brought about by the injection of heterologous protein substances into the specifically sensitized animal, may, in certain instances, when the complex and mixed proteins, such as animal sera, are used for sensitization, depend upon some type of group reaction in which the antibodies in the cells are saturated or neutralized by fractions of heterologous proteins employed. It is also possible to conceive of these heterologous proteins as interfering, in some way, with the union of the specific antigen and the specific cellular antibody. This may be a phenomenon which is analogous to the interference which an injection of large quantities of heterologous proteins has upon the specific sensitization of the animal tissues by the subsequent injection of a single specific antigen.

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## ANHYDREMIA

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When the amount of water eliminated from the body exceeds the amount ingested, plus that produced through metabolic processes, a certain degree of desiccation of the various organs, tissues and fluids occurs. The body possesses an available store of water which exists for the greater part in the muscles and in the skin, and which can be drawn upon to some extent before any considerable degree of desiccation of other parts of the body occurs. As larger amounts of water are withdrawn a drying out of all parts of the organism takes place, but the degree of desiccation varies markedly in the different organs. The fatty tissues, brain, heart and skeleton lose relatively little water as compared with the muscles skin and blood. (Falk and Scheffer, Volkmann, Nothwang, Straub, Durig, Engels, Tobler.) The muscles which compose 42.8 per cent of the body weight give up 67.89 per cent of the total water lost (Engels). The withdrawal of fairly large amounts of water from the muscles (10 to 20 per cent of their total water) fails to impair their function, as determined by electro-motor activity of the excised muscles (Durig), nor is there any apparent morphological change (Durig, Straub). The skin also seems to be but little damaged functionally or structurally by the loss of a considerable portion of its water content. In the case of the blood, conditions are quite different. Here desiccation to even a slight degree results in impairment of the circulation and, as a result, to secondary functional disturbances of almost every part of the body. There is an alteration in the metabolic processes and, when sufficient concentration of the blood occurs, a disturbance of the heat-regulating mechanism. As most of the observed physiological effects of loss of water are referable to the concentration of the blood, and as the condition of the blood serves as a fair index of the degree of dehydration of the body as a whole the subject of desiccation in general may very well be considered from the standpoint of desiccation of the blood, or *anhydremia*.

*The experimental production of anhydremia.* Anhydremia may be brought about by restricting the water intake or by increasing the output through various channels.

Some animals, for example, moths, serpents and camels, can exist for very long periods without any intake of water. Other animals under the conditions of hibernation live without water intake for long periods. Most animals, however, when living under otherwise normal conditions develop anhydremia within a relatively short time when deprived of water. When a marked degree of anhydremia is brought about death occurs. A man has been known to survive as long as 18 days without food or water when under average conditions of temperature and humidity. Under the same conditions dogs ordinarily live from 14 to 66 days when deprived of all food and water (Keith, Poleyeff, Underhill); rabbits live from 14 to 17 days (Uthlein); pigeons usually die within 6 days (Nothwang).

Ingested fluid does not represent the sole available source of water of the body, as appreciable amounts of water are produced during the processes of metabolism. When carbohydrate and fats are burned in the body, all of the hydrogen is converted into water as is also a considerable portion of the hydrogen of protein. It has been calculated by Nobel and by Magnus-Levy that 100 grams of fat give rise to 90 to 110 grams of water, 100 grams of carbohydrate to 55 to 60 grams of water and 100 grams of protein to 40 to 45 grams of water. The amount of water produced in this way serves in part to supply the needs of the body, but in the case of most animals is in itself entirely insufficient. When animals take neither food nor water by mouth a utilization of stored glycogen and fat occurs, there is also some destruction of body protein. In this process a certain amount of loosely bound water is liberated. One gram of glycogen as it exists in the body is combined loosely with about 4 grams of water. Protein is united with approximately the same amount of water. Fatty tissues are relatively dry, there being only about 0.2 gram of water to each gram of fat (Bozenraad). All of this loosely combined water is liberated and becomes available when the protein, carbohydrate and fat of the body are "burned" to supply the needs of the starving organism.

In warm-blooded animals life cannot be maintained unless there is a constant elimination of a considerable amount of water and this elimination takes place even though it may necessitate a desiccation of the body tissues and fluids. Loss of water by evaporation from the respiratory tract and mouth is inevitable unless the air breathed in is saturated with water vapor at body temperature. Evaporation brought about in this way serves the useful purpose of dissipating a part of the body heat. A somewhat larger portion of the body heat

is usually dissipated by evaporation of water from the skin. The amount thus evaporated varies tremendously according to the environment. When the external temperature is equal to that of the body ( $98.6^{\circ}$ ) or above it, no heat is lost by conduction or radiation and the only means available to dissipate the heat of metabolism is that of water evaporation. Hunt has calculated that an adult with a metabolism of 3500 calories a day would, at an environmental temperature of  $37^{\circ}\text{C}$ . ( $98.6^{\circ}\text{F}$ .), need to evaporate at least 8 liters of water daily to eliminate the heat produced, and to maintain a normal body temperature. This figure makes no allowance for sweat secreted but not evaporated, nor does it allow for the effects of active exercise. Hunt actually found that Europeans in Central India, during the summer season, usually suffered from thirst when the daily water intake fell much below 13 liters a day (200 cc. per kilo per day). Almost all of this water was eliminated from the skin and lungs as the urine volume was small.

The evaporation of water necessary under normal conditions of environment is considerably less than the above. The average adult under average conditions of temperature ( $65\text{--}70^{\circ}\text{F}$ .) and humidity (35–60 per cent) and while doing light work loses from 30 to 60 grams of water per hour in this way (Benedict and Carpenter, Sonderstrom and Du Bois, Wolpert). This corresponds to from 10 to 20 grams per kilo of body weight per day, or a total of 650 to 1400 cc. daily.

A certain amount of water is required for urinary excretion of waste products, and this amount varies considerably with the character of food material consumed. A sufficient amount of water is excreted to hold in solution urea, salts and other end products of metabolism in a concentration not too great to be capable of secretion by the kidneys. It has been shown by Ambard and Papin and by Adolph that there is a definite maximum concentration of urea and of sodium chloride that can be excreted by the kidneys of each individual, but that the excretion of each substance is quite independent of the excretion of any other. The elimination of 45 grams of urea, or 15 grams of sodium chloride in man requires the excretion of about 1 liter of water. This amount of water, however, will serve for the simultaneous excretion of both substances in the concentrations mentioned. It is thus seen that with a high protein metabolism or salt intake a proportionately large amount of water must of necessity be excreted. In man, on a general mixed diet, the daily urea excretion averages from 30 to 35 grams a day, this would require for its excretion from 650 to 800 cc. of urine per day or about 10 grams per kilo. Adolph found the urinary excretion of an

adult when abstaining from food and water to fall to the low level of 408 cc. per day which was the amount secreted even though the individual was becoming dehydrated at the time. From the above it will be seen that an adult man living under average conditions necessarily eliminates from the body in various ways from 15 to 30 grams of water per kilo per day, or a total of from 1000 to 2000 cc. per day. Under extreme conditions the amount may exceed 20,000 cc. per day. In the case of infants the evaporation of water from the skin and lungs is at least three times as great per kilo of body weight as in adults under the same environmental conditions (Rubner and Heubner). This is necessarily the case on account of the relatively higher total metabolism of the infant. The water requirements of animals for evaporation and urinary excretion vary greatly with the species.

When the intake of fluid is restricted so that this plus the potential water of metabolism is less than the amount eliminated, a certain degree of anhydremia necessarily develops in any animal. Anhydremia has been experimentally produced in man and various lower animals by simple restrictions of water in the diet (Bowin, Durig, Dennig, Engels, Falk and Scheffer, Hunt, Keith, London, Nothwang, Uthelm, Schiff, Spiegler, Straub, Underhill, Volkmann).

Dehydration of the body is much more rapidly produced if excessive elimination of water occurs at the same time that the intake is restricted. Czerny produced a high degree of anhydremia in cats by exposing them to warm dry air. Adolph observed anhydremia in man as the result of sweating in a warm room or in a warm bath. Sweating as the result of muscular exercise also favors the development of anhydremia.

Certain drugs, such as pilocarpine, bring about increased secretion of the sweat. Underhill and Roth have observed marked anhydremia in animals deprived of water and injected with pilocarpine.

Excessive removal of water from the body by diuresis results in anhydremia provided the loss is not covered by an increased fluid intake by mouth. Morgulis and Muirhead observed a considerable concentration of the blood following diuresis occasioned by cantharis injection in animals. Numerous investigators have brought about a condition of anhydremia by the administration of sodium chloride or urea by mouth and by intravenous injections of sodium chloride, urea, glucose, saccharose or lactose. All of these substances on excretion in the urine necessarily appropriate water from the body, and if this loss of water is not compensated for by additional intake anhydremia of a severe

grade is produced (Heim and John, Finkelstein, Peteri, Bingel, Straub, Balcar, Sansum and Woodyatt, Keith, Adolph).

An excessive loss of water from the body by way of the intestine follows the administration of the saline cathartics. This loss may be sufficient to lead to a considerable concentration of the blood (Underhill and Errico).

*The occurrence of anhydremia in clinical conditions.* A certain daily variation in the water content of the body necessarily occurs as the result of such factors as active muscular exercise, exposure to a warm environment or varying intervals of fluid intake. It has been shown by Grunewald and Rominger that the blood is usually somewhat more concentrated in the evening than in the morning.

Severe anhydremia occurs when there is voluntary refusal of water for prolonged periods, for instance in the case of persons mentally deranged. It occurs in individuals exposed to the heat of the desert (King, McGee) and in those working in such locations as deep mines, boiler rooms, etc. (Haldane).

Vomiting brought about from any cause results in a greatly diminished fluid intake and often to a considerable degree of anhydremia. This is especially noted in the case of infants suffering from pyloric stenosis, as no absorption of water takes place from the stomach and relatively little passes beyond the pylorus. Any form of high intestinal obstruction leads to a similar result.

In the presence of diarrhea considerable amounts of water may be lost by way of the stools. Such losses are particularly marked in Asiatic cholera and in the choleriform diarrheas of infancy. In such cases anhydremia rapidly develops and may be severe enough to lead to death in a relatively short period. The amount of water lost by the stools may be enormous and greatly exceed the amount eliminated from the body by all other ways combined (Rogers, Sellards, Meyer, Reiss, Salge, Göppert, Lederer, Lust, Rominger, Marriott, Bessau). As a result of the water loss in severe diarrhea the body weight may be decreased to the extent of 20 per cent or more within one or two days' time (Meyer, Marriott). In the types of diarrhea mentioned the factors of infection and of injury to the intestinal mucosa with a possible change in its permeability complicate the picture and make it difficult to determine to what extent the manifestations observed are due to the anhydremia *per se*.

Severe anhydremia is especially likely to occur in infants. This is due in part to the fact that they have a high water requirement which

must be covered. Furthermore the infant is entirely dependent upon others for water administration, and may be offered an insufficient amount. Newly born infants nursing at the breast and receiving no fluid from other sources frequently become somewhat desiccated due to an insufficient water intake (Crandall, Holt, Müller, Von Reuss, Uthelm, Schick, Bakwin, Aron). In any infant the presence of infection is likely to lead to refusal of food, vomiting and diarrhea, with a resulting anhydremia (Marriott).

*The composition of the blood in anhydremia.* When sudden loss of water from the body occurs the composition of the blood is much more affected than when the same loss is brought about more gradually; thus Adolph observed a concentration of the blood of 15 per cent with a 5 per cent loss of body weight when a subject was rapidly sweated in a hot bath, as contrasted with a blood concentration of only 2 per cent when the same subject lost the same amount of fluid during a period of three hours' sweating in a warm room. Keith observed a concentration of blood plasma of from 24 to 44 per cent and a total blood volume decrease of from 2 to 38 per cent when dogs were rapidly dehydrated by intravenous injections of saccharose solutions, a considerably greater concentration of the blood than is ordinarily observed following a similar decrease in body weight brought about by a more gradual water loss. Sudden withdrawal of fluid from the body results in an immediate concentration of the blood. Later water is given up from the reserve stores in muscle and skin and the blood tends to return to its normal composition providing there is not a continued removal of an excess amount of water from the body (Underhill and Errico). After a period of dehydration the administration of water causes an immediate dilution of the blood and an abrupt fall in the concentration of the hemoglobin (Underhill and Kapsinow, Keith, Rominger). The blood concentration then gradually increases, the excess of water being in part excreted by the urine and in part taken up by the more desiccated tissues. Ultimately if water continues to be administered a condition of equilibrium is finally attained between the tissues and blood after which the concentration of the blood remains constant (Hunt).

Loss of water from the blood necessarily results in an increase in the proportion of the total solids and in the dried weight of a unit volume of blood (Rominger). The specific gravity is increased. There is an increase in the red blood cell count and a corresponding increase in the hemoglobin percentage. The red blood cell count may be almost

twice as high as the normal; thus Czerny observed a count of 10,720,000 red cells in cats made anhydremic by exposure to warm dry air. Bowin also observed a red blood cell count twice that of the normal animal. Underhill and Kapsinow observed an increase in hemoglobin concentration to over 140 per cent of the normal in dogs deprived of water.

Changes in the concentration of the serum proteins of the blood are usually more marked than the red cell concentration as it is from the serum that most of the water is lost (Keith). The serum protein not infrequently increases over 50 per cent in concentration (Reiss, Marriott) a 100 per cent increase has been observed by Behrend and Tenzer in the case of a young infant (serum protein of 11.6 per cent as compared with the normal of less than 6 per cent). Serum protein determinations are readily made by the use of the refractometer (Reiss).

Such high concentrations of serum protein, hemoglobin and red blood cells are usually seen only when anhydremia is suddenly brought about. When a condition of severe anhydremia has lasted for a number of days a decrease in the concentration of hemoglobin and of serum protein occurs even though the body weight and the blood volume determinations may indicate a further loss of water (Lust, Marriott). This may be taken as indication of destruction of blood corpuscles and of serum protein. The experiments of Gürber on frogs, of Utheim on rabbits and Keith on dehydrated dogs show a decrease in the total number of red blood cells in the circulation, when the diminished total blood volume is considered in connection with the cell counts and protein concentration. As a result of this destruction of the blood constituents an abnormally low cell count, hemoglobin and serum protein content of the blood is often observed following a restoration of the blood volume by fluid administration. It is thus seen that determination of the cell count, hemoglobin or serum protein may at times fail to indicate accurately the degree of anhydremia. The same may be said of the determination of the total solids. The measurement of the blood volume taken together with the determination of the other constituents mentioned supplies the necessary data for the estimation of the degree of anhydremia.

The mineral salts of the blood usually show much less change in concentration than does the protein. When the blood plasma becomes concentrated through water loss the inorganic salts are excreted in the urine in such amounts as to maintain an approximately normal salt concentration of the plasma in most instances (Heim and John, Salge, Reiss, Rogers). Some increase in salt concentration of the plasma occurs

when anhydremia is brought about suddenly and also when the anhydremia is of such a degree that renal excretion is greatly impaired. Keith observed a moderate increase of the chlorides of the serum in severe anhydremia. Salge found some increase in the electrical conductivity of the serum in marked anhydremia. Of the individual inorganic salts, phosphates are more likely to be retained than chlorides, when renal activity diminishes as the result of blood concentration (Howland and Marriott). The freezing point of the blood, according to Salge, is lowered, but he attributes this to the increased concentration of organic constituents rather than inorganic salts.

As the result of the concentration of the blood protein and cellular elements the viscosity of the whole blood is greatly increased (Keith, Behrend and Tenzer, Czerny, Lust). The increase in blood viscosity is readily apparent, the blood appears thick and at times syrupy and does not flow readily. McGee states that in a man severely anhydremic as the result of exposure to desert heat, no bleeding occurred from deep cuts on the body.

The total non-protein nitrogen as well as the urea of the blood is high when any considerable degree of anhydremia occurs (Schloss and Stetson, Bang, Minsk and Sauer, Bessau, Valk and DeLangen). A blood non-protein nitrogen as high as 590 mgm. has been observed in cases of Asiatic cholera (Valk and DeLangen). The high non-protein nitrogen of the blood is to be explained in part by the functional disturbance of the kidney as the result of dehydration of the blood and in part by the increased destruction of body protein which is known to occur.

The blood sugar is often higher than normal, thus Schloss observed fasting blood sugar of 0.14 to 0.20 per cent in the case of infants anhydremic as the result of severe diarrhea. Even higher figures have been found recently by the present author. High blood sugar is a common finding in other conditions associated with a low blood volume, for example, in shock (Cannon). It is also observed in many conditions in which there is vasoconstriction or a diminution of the oxygen-carrying capacity of the blood and an impairment of the circulation (Araki). As will be seen later, anhydremia results in a decreased blood volume and an impaired circulation and oxygen-carrying capacity in the blood and vasoconstriction.

The blood often contains an abnormal amount of lactic acid (Clausen). Acetone, diacetic acid and oxybutyric acid may be present, but rarely in excessive amounts (Moore). Despite the fact that large amounts of

organic acid are not found in the blood there are changes which are indicative of a considerable degree of acidosis. There is a diminished alkali reserve, carbon dioxide content and bicarbonate-combining power of the blood. The dried residue of the serum is more acid than normal. There is a decreased oxygen capacity of the hemoglobin and an increased hydrogen ion concentration of the venous blood (Sellards, Howland and Marriott, Ylppö, Schloss and Stetson). The cause of the acidosis will be discussed subsequently.

*The blood volume in anhydremia.* As a result of the withdrawal of water from the blood the volume necessarily decreases (Rogers, Czerny, Marriott, Uthelm, Keith). The reduction in volume may amount to 40 per cent or more of the total and this is due mostly to a decrease in plasma volume (Rogers, Keith). The volume of the blood is rapidly regained when water is administered, provided the anhydremia has been of a slight degree or of short duration, but is only slowly regained when a severe degree of anhydremia has persisted for a considerable time. The blood volume does not return to normal until regeneration of the destroyed cells and protein has taken place or until these constituents have been restored by blood transfusion (Uthelm, Keith, Marriott).

*The circulatory system in anhydremia.* With desiccation of the blood and reduction in its volume there occurs a compensatory constriction of the peripheral arterioles and a greatly decreased volume flow of the blood through certain parts of the body. The changes in the circulation are essentially the same as those occurring in other conditions associated with a diminished blood volume (shock, hemorrhage, etc.). The constriction of the peripheral arterioles leads to a stagnation of the corpuscles in the smaller peripheral vessels. On this account cell counts and hemoglobin determinations made on blood obtained by puncture of the skin are considerably higher than on blood simultaneously obtained from the veins of the same individual. A difference of 25 per cent in the counts between the venous and capillary blood has been observed in the blood counts in the case of infants with severe diarrhea (Marriott, Uthelm). These differences are similar to those observed by Cannon, Fraser and Hooper in soldiers suffering from wound shock.

Another effect of diminished blood volume is a greatly decreased volume flow of the blood through the extremities. The flow of blood per unit volume of extremity per minute was found to be less than 10 per cent of the normal flow, in the case of anhydremic infants (Marriott, Uthelm). Similar changes in the circulation were observed by Uthelm

in rabbits and dogs deprived of water for a considerable time. The measurements of volume flow were made by means of the Stewart calorimetric method, and confirmed in the case of dogs by the use of the Ludwig "Stromuhr." The volume flow of the blood through the extremities increases when the volume of the blood is restored (Marriott, Uthelm). It is not known to what extent the volume flow of the blood through the internal organs is diminished. The diminished flow in the extremities would tend to compensate for changes in blood volume and to maintain the circulation through the more vital portions of the body (Gesell).

Disturbances in the cardiac mechanism have been observed by McCulloch in the case of anhydremic infants, the electrocardiograms showing a very low amplitude of all waves, the "T" waves being entirely absent. The P-R interval was increased to as much as 0.20 second, a distinctly prolonged interval for an infant. The Q. R. S. complexes were abnormal in form. Following a restoration of normal blood volume and volume flow the electrocardiograms became normal. This latter finding would support the idea that the cardiac involvement is purely functional and likely the result of an impaired circulation through the coronary vessels. Additional support for this supposition is furnished by the fact that the changes in the electrocardiograms are very similar to those observed in conditions known to be associated with an impaired circulation through the coronary arteries, for example, in coronary sclerosis (McCulloch). This disturbance in the cardiac mechanism may well be a factor in impairing the general circulation.

The pulse becomes small and rapid as anhydremia develops.

The blood pressure is often well maintained, even when a very considerable degree of anhydremia occurs (Feilchenfeld, Uthelm, Keith). The increased viscosity of the blood is presumably an important factor in preventing the fall of blood pressure which might otherwise be expected to occur as a result of decreased blood volume. Keith found a normal blood pressure in dogs rapidly dehydrated by saccharose injection, but a lowered blood pressure in animals rendered anhydremic by a prolonged period of abstinence from food and water. It is known that in the latter case the viscosity of the blood is not so high, due to the destruction of blood cells and serum protein (see preceding discussion of composition of the blood in anhydremia). Somewhat lowered blood pressures have been observed by Dennig in man following a restricted water intake. Rogers states that the blood pressure is often very low as the result of anhydremia due to Asiatic cholera.

*The urine and renal function in anhydremia.* Starling has shown that the secretion of the urine is greatly decreased as the colloidal osmotic pressure of the blood approaches the arterial pressure in the renal arterioles. Such a condition exists when the blood becomes concentrated by water loss, the secretion of urine is, therefore, greatly diminished as the result of anhydremia. There may even be complete anuria. Such urine as is secreted has a high specific gravity, 1040 or above (Bessau, Spiegler).

Traces of albumin and numerous casts are regularly present.

The urine contains small amounts of reducing sugars (Schiff, Langstein and Steinitz, Schloss). It has been shown by Schloss that the sugar of the urine is chiefly glucose, its presence being readily explained as the natural result of the hyperglycemia previously referred to. In addition to glucose, lactose may appear in the case of anhydremic infants (Langstein and Steinitz, Schloss). The excretion of lactose indicates the absorption of unsplit lactose through the intestinal wall. Bessau states that anhydremia leads to an increased permeability of the gastro-intestinal mucosa. There is but little experimental evidence that the increased permeability is due to the anhydremia *per se*.

There is an excess of organic acids in the urine (Utheim, Clausen). The larger part of this acid is insoluble in ether and contains nitrogen. Its exact nature is unknown but in chemical behavior it resembles closely "oxypoteic acid" (Clausen, Utheim). In the ether soluble fraction of the organic acids, a moderate amount of lactic acid is present (Clausen). There is rarely a significant amount of beta-oxybutyric or diacetic acids (Sellards, Howland and Marriott, Schloss and Stetson).

There is evidence of a distinctly impaired functional capacity of the kidney. Mention has already been made of the fact that the blood shows an excess of urea, total non-protein nitrogen and inorganic phosphate. The phenolsulphonephthalein excretion is lower than normal and the Ambard coefficient is high (Schloss and Stetson). The renal insufficiency is not to be explained on the basis of a definite nephritis as the kidney only occasionally shows pathological changes at autopsy (Schloss, Bessau). Furthermore, all evidences of functional renal impairment rapidly disappear with the establishment of a normal water balance. The urine becomes normal in all respects. These facts lead to the conclusion that the renal insufficiency is purely functional and the result of the inability of the kidney to separate a normal urine from a concentrated blood.

*The effect of anhydremia on the metabolism.* Straub found no increase in the total metabolism of dogs made anhydremic by water deprivation.

Numerous observers have established the fact that anhydremia causes a negative nitrogen balance (Dennig, Straub, Meyer, Landauer, Schiff, Spiegler). This indicates a destruction of body protein.

The mineral salt balance is also negative. There is a loss of sodium, potassium and chloride ions (Meyer, Tobler).

*The gastro-intestinal tract.* One of the effects of anhydremia is a poor absorption of fat and protein from the gastro-intestinal tract (Dennig, Gürber). When food is given to a badly desiccated animal, vomiting and diarrhea usually occur (Straub, Nobel, Heim and John, King, McGee, Marriott).

*Acidosis.* Desiccation of the body leads to a considerable degree of acidosis. The blood shows characteristic evidences of acidosis, as has been mentioned above. In addition there is a lowered carbon dioxide tension in the alveolar air (Haldane, Howland and Marriott, Schloss and Stetson), and an increased alkali "tolerance," that is to say, the capacity to ingest large amounts of alkali without changing the reaction of the urine to the alkaline side (Sellards, Howland and Marriott, Schloss and Stetson).

The acidosis is not due to an excessive production of the acetone bodies (Howland and Marriott, Moore, Schloss and Stetson). It is due in part to the acids produced as the result of a diminished volume flow of the blood. It has been shown by Wright and Colebrook, by Gesell and others that a diminished volume flow of the blood leads to an overproduction of acids in the tissues, presumably as the result of suboxidation. Clausen has found two of such acid products in the blood and urine of desiccated animals, namely, lactic acid and an ether-insoluble acid resembling oxyproteic acid. Both of these disappear with the establishment of the normal blood flow.

When the urine secretion is much diminished as the result of anhydremia, there is a retention of acids normally excreted. The predominating acid of the urine is ordinarily acid phosphate. In severe anhydremia a phosphate retention occurs (Howland and Marriott). This retention of acid is an additional factor in the production of acidosis. The acidosis is likely of the same general nature as that occurring as the result of chronic nephritis (Marriott and Howland).

*The heat-regulating mechanism.* When the blood and tissues become concentrated by water loss the amount of water available for evaporation is diminished and ultimately becomes less than that required for

removal of the heat of metabolism. Fever then occurs. High body temperatures in man ( $104^{\circ}\text{F}$ . or over) as the result of restricted fluid intake have been observed by Dennig, Rosenstern, Jurgenson, Von Noorden and Solomon. McGee mentions fever as one of the prominent symptoms of desert thirst. Aron, Friese, Grulee and Bonar and Meyer have observed high temperatures in infants of various ages when receiving less than the usual amounts of water. In these cases no cause for fever other than the restricted fluid intake could be found. The temperature usually fell promptly on the administration of water.

Infants during the first days of life occasionally develop high fever which is lowered by administration of water. Such infants are often receiving but small amounts of fluid and show definite evidences of anhydremia (Holt, Crandall, Müller, Rott, Heller, Bakwin, Schick, Von Reuss). It is possible however that the anhydremia is not the sole cause of fever in these infants (Grulee, Utheim, Von Reuss).

Fever has been observed in animals rendered anhydremic by the administration of salts and sugars (Finkelstein, Heim and John, Peteri, Balcar, Sansum and Woodyatt). Temperatures as high as  $125^{\circ}\text{F}$ . have been observed under such conditions. There is some question as to whether the fever observed (salt fever) is solely the effect of dehydration.

Czerny observed high body temperatures in cats made anhydremic by exposure to warm dry air.

*Miscellaneous manifestations of anhydremia.* During the development of anhydremia loss of weight occurs which is more rapid than that observed in any other condition. From 10 to 25 per cent of the body weight may be lost within 1 or 2 days' time. The skin becomes gray, wrinkled and dry and loses its elasticity. A fold produced in the skin flattens out slowly. The surface of the body may become anesthetic. The mucous membranes are dry and lustreless. Salivary secretion ceases. The tongue and lips are shrivelled. In infants the fontanelle is depressed.

The extremities are cold even though the rectal temperature may be high. The respirations are deep and often stertorous. This deep breathing, or "air hunger," is a manifestation of acidosis.

Transient deafness and blindness have been observed. The mental state is one of irritability during the earlier stages of anhydremia, later one of stupor. Terminal convulsions are not infrequent. The nervous symptoms are in part referable to the uremia, the result of impaired renal function.

Excellent descriptions of the symptoms of anhydremia in man resulting from desert thirst are given by King and by McGee.

London states that anhydremia lowers the resistance of the body to infection.

*The treatment of anhydremia.* Administration of water by mouth in sufficient amounts leads to the prompt disappearance of all the manifestations of anhydremia providing the degree of desiccation is not extreme, and has not lasted long enough to have led to much destruction of the body constituents. In severe anhydremia administration of water by mouth in sufficient amounts is often difficult as vomiting is likely to occur. Water is well absorbed through the skin of certain animals, such as frogs. McGee states that the human skin when desiccated is capable of absorbing water applied externally. Definite proof of this assertion is, however, lacking.

Water introduced by way of the rectum is well absorbed.

Fluid in the form of isotonic salt and glucose solution may be introduced intravenously, subcutaneously or intraperitoneally (Rogers, Blackfan and Maxey).

As there is a loss of mineral salts from the body in anhydremia there is an advantage in giving salts as well as water. It has been found that water is much better retained in the body when salts are given at the same time (Czerny, Heim and John, Rogers, Behrend and Tenzer).

As all of the manifestations of anhydremia including the acidosis rapidly disappear when sufficient fluid is administered it is usually unnecessary to give alkali to combat the acidosis. Administration of sodium bicarbonate may even be harmful on account of its effect on further concentrating the blood and because of the alkalosis which usually occurs as a normal water balance of the body is established following anhydremia. The organic acids are burned and excreted leaving behind the excess of alkali which may be slowly excreted over several days. The excess of sodium bicarbonate in the body occasionally leads to the development of manifestations of tetany (Harrop, Marriott).

When anhydremia has existed for a considerable time so that there is a destruction of blood cells and plasma protein the administration of fluid may fail to restore the blood to its normal volume and as a consequence the volume flow of the blood will remain low for some time. This condition may be remedied by transfusion of whole blood, or to a certain extent, by the injection of solutions containing acacia (White and Erlanger, Gesell, Marriott).

*Appendix: The blood and the circulation in conditions closely related to anhydremia.* In traumatic shock and in experimental shock brought about by mechanical means or by the injection of histamine or proteoses a decrease in the blood volume occurs (Cannon, Keith, Gasser; Erlanger and Meek, Underhill and Ringer, Underhill and Roth). The concentration of hemoglobin and of red blood cells is markedly increased. The total solids of the blood as a whole are increased but the plasma protein concentration remains approximately normal. There is no actual desiccation of the blood, but a loss of plasma presumably by passage through the vessel walls. A similar concentration of cellular elements of the blood occurs in influenza (Underhill and Ringer). In intestinal obstruction (Whipple and Cooke) and following severe burns of the surface of the body (Underhill) and the inhalation of war gases (Underhill) a similar blood picture is produced. In all of the conditions mentioned there is a decrease in blood volume and changes in the circulation which are essentially the same as those observed in anhydremia. Acidosis, hyperglycemia and an increase in the urea and non-protein nitrogen and lactic acid of the blood have been observed in some of these conditions (Aub and Wu, Cannon, Macleod).

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## NEUTRALITY REGULATIONS IN THE BODY

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VARIATIONS IN THE REACTION OF THE BLOOD. The normal processes taking place in the living cells can be carried out only if the hydrogen ion concentration of the fluids inside and outside of the cells is maintained constant within quite narrow limits. The blood in the body is almost always slightly alkaline (about pH 7.4). Although the limits of variation in reaction within which life is possible are still in doubt, blood as acid as pH 6.95 (Hasselbalch and Lundsgaard, 1912; Van Slyke, Austin and Cullen, 1922) and as alkaline as pH 9.00 (Dale and Evans, 1922) has been reported. In spite of the great amount of work which has been done in this field there are but few data available which may be compared indiscriminately owing to the technical difficulties involved in determining the hydrogen ion concentration (cH) of blood by the electrometric method in the presence of oxygen and carbon dioxide, and owing to the lack of accurate constants for use in calculations where indirect methods are employed.

The limits of variation in the normal individual which result from temporarily taxing the available mechanisms for maintaining neutrality are nearly as great as the extremes just mentioned. Barr, Hinwich and Green (1923) reported a pH of 7.05 (recalculated)<sup>1</sup> in blood after short periods of strenuous muscular exercise. On the alkaline side, Davies, Haldane and Kennaway (1920) found a pH of about 7.85 (recalculated) after forced breathing.

The difference in pH between arterial and venous blood has been studied by several investigators. Parsons (1917) calculated the change in pH as 0.02. Peters, Barr and Rule's (1920-21) data show variations

<sup>1</sup> Data designated in this way have been recalculated using the "ordinate correction" of Doisy, Briggs, Eaton and Chambers (1922) and 6.15 as the value of  $pK_1$  in Hasselbalch's equation.

of 0.01 to 0.04 (recalculated). Doisy, Briggs, Eaton and Chambers (1922) calculated variations from 0.013 to 0.037. Dale and Evans (1922) using their colorimetric method, found a difference of 0.10 between the arterial and venous blood of an anesthetized cat.

**THE REACTION OF CELL FLUIDS.** These figures are for blood only and they tell us nothing about the reaction of the tissue cells. The study of the reaction of the fluids within the tissues is difficult on account of the rapid changes which occur when the tissue is removed from its normal environment. It appears, however, that the reactions of the blood and tissue cells are not widely different (Kochler, Severinghaus and Bradley, 1922).

One might suppose, at first glance, that the study of cells, such as those of the stomach which secrete hydrochloric acid, would furnish some clue as to the degree of acidity which these cells are able to withstand. Gastric juice has a pH of 1.7 and is the most acid fluid found in the body. Comparing the work of Fitzgerald (1910), Harvey and Bensley (1912) and Collip (1921) one gains the impression that within the parietal cells hydrochloric acid is formed (by the interaction of chlorides, phosphates and carbonic acid), which unites with a weak base and the resulting salt (seemingly of colloidal nature) yields a solution which is only faintly acid. The compound passes into the lumen of the excretory duct and, as it passes toward the opening into the stomach, is hydrolyzed and the weak base reabsorbed. The final acidity is reached only as the liquid is about to flow out into the stomach, where the cells are protected by a mucous coating. The contents of the secreting cells are, however, never more than very faintly acid. Some such mechanism involving selective diffusion may come into play in the mammalian pancreas and liver, where strongly alkaline fluids are secreted and may even occur in connection with the formation of urine by the kidney. These secretions of specialized cells offer opportunities for studying certain physico-chemical reactions, such as selective permeability through membranes, but do not necessarily furnish any information concerning the variations of the reactions within the tissue cells themselves.

The most satisfactory comparison of pH inside and outside of cells has been made in studying plasma and corpuscles. Fluid within the corpuscles has been found to be more acid than the plasma by about pH 0.05 to 0.13 (for references see below). That the pH within the cells may be slightly different from the blood which bathes them is made possible by the fact that certain substances are not free to diffuse

through the cell membrane. Nevertheless, an equilibrium exists between the  $\text{cH}$  of the cell fluid and that of the blood so that an alteration of one will be reflected in the other. The maintenance of a normal hydrogen ion concentration of the blood is, therefore, necessary in order that the activities of the cells shall not be impeded by abnormal changes in their reaction. But, besides carrying out this important function, the normal reaction of the blood must be maintained in order that it may act as an efficient carrier of oxygen and carbon dioxide.

**MECHANISMS FOR REMOVING BASE AND ACID.** Since the normal reaction of the body is maintained within such narrow limits there must be effective mechanisms within the body for taking care of a temporary excess of either base or acid, and since acids are formed in many phases of metabolism the organism evidently must have considerable capacity for handling acids. In the oxidation of food materials to furnish the energy and heat of the body, proteins, fats and carbohydrates all furnish large quantities of carbonic acid, which, although a weak acid, would cause serious injury to the tissues if allowed to accumulate in too high a concentration. Besides yielding carbonic acid upon oxidation proteins yield appreciable quantities of phosphoric and sulphuric acid. After strenuous muscular activity lactic acid accumulates. Under abnormal conditions beta-oxybutyric acid and aceto-acetic acid may be formed in large quantities. Thus it may be seen that the animal organism is called upon to dispose of large amounts of acid under both normal and abnormal conditions and to dispose of them sufficiently rapidly so that the hydrogen ion concentration of the blood and tissues does not rise above  $1 \times 10^{-6.9}$ . The disposal of basic radicles (mainly sodium, potassium, calcium and magnesium) is necessitated by the fact that salts containing basic radicles are a necessary part of the normal diet and the accumulation of an excess of base in the body would result in increased alkalinity of the tissue fluids as well as severe disturbances in the osmotic pressure equilibrium.

The great importance of regulating the reaction of the body within narrow limits is indicated by the number of elaborate mechanisms available for maintaining a normal acid-base equilibrium. Acids and bases which tend to accumulate in the body are excreted. The volatile  $\text{CO}_2$  is eliminated by way of the lungs while the non-volatile acids, such as sulphuric, phosphoric and hydrochloric, and the bases are eliminated by way of the kidneys in the form of salts. Some salts probably pass from the blood into the intestine and are eliminated in the feces.

The tissues and blood must be protected against any great change in reaction during the period between formation and elimination of acid. The buffers in the tissues and blood constitute the great source of protection in this connection. The proteins and phosphates are probably the main buffers concerned.

It should be emphasized that the  $\text{NaHCO}_3$  of the blood is the agent which neutralizes non-volatile acids such as sulphuric and phosphoric. Upon entering the blood, they react with  $\text{NaHCO}_3$  as they would in a test tube, forming the corresponding salt of the non-volatile acid and liberating carbon dioxide. In a test tube the gas quickly passes from the solution into the air and, in a similar way in the body, the extra carbon dioxide is eliminated by the lungs and thus the end result is that the  $\text{NaHCO}_3$  in the blood has neutralized a strong acid with no appreciable change in the pH of the solution. The neutralization of carbonic acid itself is brought about, not by the  $\text{NaHCO}_3$  of the blood, but by protein and phosphates which, acting as storehouses for base, give it up thus permitting the formation of bicarbonate with the carbonic acid as it enters from the tissues.

In tracing the method of disposal of acid or base which has accumulated in some tissue, the following mechanisms have been shown to be concerned. The buffers of the tissue protect while the material diffuses into the blood and adjacent tissue. The material is carried by the blood with little change in reaction owing to its buffers. Other tissues of the body absorb some of the excess from the blood, so that the quantity of acid or alkali which the body can retain without exceeding the normal limits of capacity of the buffers depends not only upon the quantity which may be handled by the blood but also by the tissue fluids of the whole body. (Palmer and Van Slyke, 1917; Barr and Hinwich, 1923, a.) This safeguards the body against an accumulation of material in excess of its ability to immediately excrete it. Besides this removal of excess acid and alkali by the body fluids, the tissues are able to destroy certain bases and acids or sometimes to form compounds to neutralize them. Ammonia, which is produced by the deaminization of amino acids, is quickly converted into the neutral compound, urea, while many organic acids are oxidized to carbonic acid. Ammonia is formed by the kidney (Nash and Benedict, 1922) to combine with excess acidic radicals, and lactic acid may be formed to combine with an excess of base (Macleod and Knapp, 1918-19). Besides these chemical mechanisms which aid in handling excess acids or bases until they are excreted, a physical mechanism of fundamental importance is the blood flow, the

rate and volume of which determine the amount of material which can be removed from a localized area and transported to the excretory organs.

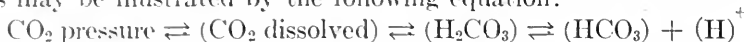
There are several possible paths for excretion of acids and bases. The quantities excreted in the perspiration are too small to warrant consideration. The excretion by way of the feces seems to have little relation to the acid-base equilibrium in the body. We may say then that the volatile carbonic acid formed in the body is excreted by way of the lungs, and the kidneys constitute the only other regulated mechanism for the elimination of acids and bases. The relative quantities of acids removed by these two paths are so seldom emphasized that it is of interest to note that the equivalent of 20 to 40 liters of normal acid are excreted by way of the lungs in 24 hours, while during the same time 50 to 150 cc. of normal acid are excreted by way of the kidneys. Not only is by far the larger part of the acid formed in the body eliminated by way of the lungs but it is eliminated without the loss of any base from the body.

**THE ELIMINATION OF CARBONIC ACID.** Stated in its simplest form, the elimination of carbonic acid depends on the diffusion of its anhydride, carbon dioxide, from the blood into the alveoli and lower air spaces of the lungs which are being washed out by an intermittent inspiration of atmospheric air and expiration of this air mixed with alveolar air which has diffused into it. Efficient aeration of the blood is made possible by a capillary network over the alveoli which permits a film of blood to come into close contact with the air in the alveoli, separated only by a very thin layer of tissue (said to be 0.004 mm. thick). The normal contact between air and blood seems to be sufficiently intimate to permit rapid diffusion of carbon dioxide and practically complete equalization of the partial pressure of carbon dioxide in the two media (Krogh and Krogh, 1909-10). There is about 0.04 per cent  $\text{CO}_2$  in atmospheric air so that the partial pressure of  $\text{CO}_2$  is about 0.3 mm. Hg. The normal partial pressure of  $\text{CO}_2$  in the alveolar air and arterial blood is about 40 mm. Hg. It would appear that under normal conditions the aeration of the blood is dependent mainly upon the degree of aeration of the alveolar air spaces rather than upon the rate of diffusion of carbon dioxide from the blood into the alveoli.<sup>2</sup> It is quite probable that all of the alveolar air spaces of the lung are not being uniformly ventilated so that the partial pressure of  $\text{CO}_2$  is higher in some regions than in others.

<sup>2</sup> Under certain pathological conditions these relations may not exist.

The blood flowing through various parts of the lung will, therefore, be brought into equilibrium with the different  $\text{CO}_2$  tensions. For this reason it is best to say that the respiration is regulated so as to maintain a certain average tension of  $\text{CO}_2$  in the alveoli which will produce in the mixed arterial blood flowing from the lung, a  $\text{cH}$  just sufficient to furnish the normal stimulus to the respiratory center (Haldane, 1922).

It is important to emphasize that even with a constant  $\text{cH}$  in arterial blood the partial pressure of  $\text{CO}_2$  in blood and alveolar air may vary. This may be illustrated by the following equation:



which indicates that, with  $\text{HCO}_3$  present in blood, there must also be present some molecules of  $\text{H}_2\text{CO}_3$  and molecules of dissolved  $\text{CO}_2$  gas and a tendency for this gas to escape from the solution. This tendency can be expressed as partial pressure of  $\text{CO}_2$  and will be demonstrated by the passage of  $\text{CO}_2$  from the blood into air with which it is brought in contact, unless the partial pressure of  $\text{CO}_2$  in the air is as great as the partial pressure of  $\text{CO}_2$  in the blood.

If for any reason the concentration of  $\text{HCO}_3$  ions in the blood is decreased (as in diabetic acidosis) and the  $\text{cH}$  remains constant, there must be a corresponding decrease in concentration of each member of the above series, and a decrease in partial pressure of  $\text{CO}_2$  in the blood. This will result in a decreased tension and a decreased percentage of  $\text{CO}_2$  in the alveolar air. With the normal aeration of the alveolar air spaces the amount of  $\text{CO}_2$  removed will be correspondingly smaller than normal. Therefore, with decreased  $\text{NaHCO}_3$  in the blood, an increased ventilation will be necessary to cause the removal of a constant quantity of  $\text{CO}_2$  formed by the oxidations in the body and at the same time to maintain a normal  $\text{cH}$ . With a high bicarbonate concentration in the blood, the alveolar  $\text{CO}_2$  tension will be high if the  $\text{cH}$  remains normal and a diminished ventilation of the lungs will suffice to remove the necessary quantities of  $\text{CO}_2$ .

Increase in the frequency of respiration will increase the aeration of the active air spaces, i.e., the air spaces which are being well ventilated. An increase in the depth of respiration will increase the number or volume of air spaces being well ventilated. Each of these mechanisms together with an increase in blood flow through the lungs will permit the removal of increasing quantities of  $\text{CO}_2$  from the body. Alterations of these three variables, therefore, offer almost unlimited opportunity for delicate adjustments whereby the loss of carbonic acid from the body can be controlled within very narrow limits.

**CHANGES OCCURRING IN THE BLOOD.** Before discussing the regulatory mechanisms which control the loss of carbonic acid, the changes which take place in the blood may be examined. Venous blood loses  $\text{CO}_2$  in the lungs and tends to become more alkaline, and arterial blood takes up  $\text{CO}_2$  in the tissues and tends to become more acid. A number of other reactions occur simultaneously which cause the changes in reaction to be diminished greatly and there results a system of transport which carries large quantities of carbonic and other acids, loading in the tissues and unloading in the lungs and kidneys with little change in  $\text{cH}$  in either place.

The study of these changes in blood has occupied the attention of many investigators for over fifty years. Recently the work has received a great impetus due to the development of new methods for determining the  $\text{cH}$  (Hasselbalch, 1911; Sørensen, 1912; Michaelis, 1914; Clark, 1922) and the oxygen and carbon dioxide concentration in blood (Barcroft, 1914; Van Slyke and Stadie, 1921; Haldane, 1922). The methods have been improved until the data are now becoming sufficiently exact to give an accurate insight into the mechanisms involved. Van Slyke and his associates (1917-23) and L. J. Henderson, (1920-21) have assembled data and proposed new and more exact mathematical methods of studying the complex reactions involved. Warburg (1922) has made a detailed application of new theories of solutions and activities of ions together with a consideration of the complications introduced by the heterogeneity of all biological fluids containing colloids and colloidal membranes, which will at least modify some of the quantitative aspects of the discussions.

Investigators are encouraged, however, to find that so much of the data may be explained within the desired limits of accuracy by a consideration of the simpler theoretical applications. The principal factors involved in the acid-base equilibrium of the blood have been summarized in an article by Van Slyke (1921a) in a previous volume of the *PHYSIOLOGICAL REVIEWS*. Since that article was published, many experiments have been carried out which furnish more exact data and extend the knowledge of these mechanisms.

**THE HEMOGLOBIN-OXYHEMOGLOBIN CHANGE.** Blood in passing through the lungs takes up oxygen until the partial pressure of oxygen dissolved in the fluid is the same (or nearly the same) as that in the alveolar air and the hemoglobin has been changed almost entirely into oxyhemoglobin. The oxygen tension in the tissues is less than that of fully oxygenated blood owing to the utilization of oxygen by the tissues

for oxidations which are going on to a greater or less extent at all times. For this reason as arterial blood passes through the tissue capillaries, oxygen leaves the blood and diffuses into the tissue cells. At the same time carbon dioxide diffuses from the tissues into the blood owing to the difference in partial pressure of the gas in the two systems. The diffusion of each gas goes on entirely independently of the other depending only on the respective diffusion constants and the variation in partial pressure of each gas in the two liquid systems. There are secondary reactions, however, which take place and have an important bearing on the neutrality regulation of the blood.

The diffusion of carbon dioxide into the blood would obviously increase the carbonic acid and cause an increase in cH of the fluid. However, as oxygen diffuses out of the blood and the oxygen tension is thereby reduced, oxyhemoglobin dissociates into oxygen and hemoglobin. The two chemical compounds, oxyhemoglobin and hemoglobin, have slightly different chemical properties. In solutions having the reaction of the blood both act like weak acids, but oxyhemoglobin is a stronger acid than hemoglobin. ( $K$  for oxyhemoglobin =  $1 \times 10^{-6.3}$ ;  $K$  for reduced hemoglobin =  $1 \times 10^{-6.9}$ .) The loss of oxygen from the blood causes some of the acid oxyhemoglobin to change into the weaker acid, hemoglobin. This is entirely analogous to a solution of hydrochloric acid suddenly being transformed to acetic acid. The resulting solution in both cases would be more alkaline. But at the time when the blood tends to become more alkaline due to the change of oxyhemoglobin to hemoglobin, carbonic acid enters from the tissues.

As all of the above reactions are equilibrium reactions, the obvious corollary is that the entrance of carbon dioxide with a resulting increase in cH will tend to increase the dissociation of oxyhemoglobin and increase of oxygen tension, thereby facilitating the diffusion of oxygen from the blood into the tissues. Exactly the reverse of this mechanism is encountered in the lungs. The entrance of oxygen into the blood with consequent increase in oxyhemoglobin tends to increase the acidity of the solution, setting free some carbon dioxide from the bicarbonates, and to accelerate the diffusion of carbon dioxide into the alveolar air.

The series of reactions taking place among the various buffer mixtures in the blood has been adequately explained in Van Slyke's paper (1921, a) on the carbon dioxide carriers. From his discussion it will be evident that the major portion of the carbon dioxide carried from the tissues to the lungs is carried in the form of bicarbonate salts of the bases present in the blood. The base is furnished by the various buffer

mixtures of the blood, mainly by the hemoglobin. The oxyhemoglobin and reduced hemoglobin exist in the blood partly as free acids and partly as salts. As oxyhemoglobin is the stronger acid the proportion of salt to free acid is greater than in the case of reduced hemoglobin, at the same cH.

$$\frac{\text{BHbO}_2}{\text{HHbO}_2} > \frac{\text{BHb}}{\text{HHb}}$$

If oxyhemoglobin changes to reduced hemoglobin, some of the base may go to form bicarbonate without change in cH of the blood. Thus, in the tissues, as oxygen leaves the blood and CO<sub>2</sub> enters, a certain quantity of CO<sub>2</sub> will be neutralized without change in reaction of the blood (isohydrically). But if more CO<sub>2</sub> enters than can be neutralized by this reaction, the cH of the blood increases with a consequent "liberation" of base from all the buffers of the blood. As a result, in all of the ratios:

$$\frac{\text{BHb}}{\text{HHb}} \quad \frac{\text{BHbO}_2}{\text{HHbO}_2} \quad \frac{\text{BBHPO}_4}{\text{BHHPO}_4} \quad \frac{\text{B protein}}{\text{H protein}}$$

the numerators will be decreased and the denominators increased.

Van Sýke (1921a) calculated the extent to which the various buffer systems play a part in carrying carbon dioxide, using data from the papers of Christiansen, Douglas and Haldane (1914) and Joffe and Poulton (1920-21) and supplying the necessary approximations at places where the data were incomplete. His calculations indicated that the total absorption of carbon dioxide due to base furnished by hemoglobin accounts for 84 per cent to 94 per cent of the total carbon dioxide absorbed with a change in pH of 0.025 to 0.09.

Recently Doisy, Briggs, Eaton and Chambers (1922) have carried out more complete experiments to obtain an evaluation of the buffers of the blood. From determinations of the CO<sub>2</sub> absorption curves of oxygenated and reduced blood and a hemoglobin solution, and determinations of inorganic phosphate, they have calculated the carbon dioxide carrying power of the various systems. Their results, recorded in the table below are valuable in yielding a closer experimental proof of the value of the various buffers and are the most exact available at present. But, owing to the numerous assumptions necessary on account of lack of available data these calculations are only close approximations.

It may now be stated without a doubt that by far the greater part of the CO<sub>2</sub> entering the blood from the tissues is carried to the lungs as the

salt of base released from hemoglobin. Half or more of the exchange takes place due to the isohydric change of oxyhemoglobin to hemoglobin, while a considerable portion of base is furnished by these two compounds acting as buffers, in a solution becoming slightly more acid due to the entrance of  $\text{CO}_2$ .

THE SHIFT OF CHLORIDES BETWEEN CORPUSCLES AND PLASMA. It is of interest to note that hemoglobin, the most important buffer in the blood, is isolated from the tissues by being placed in the corpuscles from

TABLE I

*Carbon dioxide carried by buffer systems studied (Doisy, Briggs, Eaton and Chambers 1922)*

	E. A. D.		W. H. C.		J. M.	
	Vol. per cent	Per cent of total	Vol. per cent	Per cent of total	Vol. per cent	Per cent of total
Total $\text{CO}_2$ carried for R. Q. of 0.75.....	2.32		4.23		5.08	
$\text{BHCO}_3$ carried isohydrically $\text{BHbO}_2 \rightleftharpoons \text{HHb}$ .....	1.233	53.1	2.262	53.5	2.72	53.5
$\text{BHCO}_3$ carried by change of pH						
By hemoglobin: $\text{BHbO}_2 \rightleftharpoons \text{HHbO}_2$						
$\text{BHb} \rightleftharpoons \text{HHb}$ .....	0.439	18.9	1.070	25.3	1.384	27.2
By $\text{B}_2\text{HPO}_4$ in cells.....	0.010	0.43	0.012	0.3	0.013	0.25
By separated serum.....	0.089	3.84	0.198	4.7	0.142	2.8
$\text{CO}_2$ physically dissolved.....	0.249	10.7	0.511	12.1	0.657	12.9
Sum, per cent of total.....	2.020	87.0	4.053	96.0	4.196	97.0
Per cent of total $\text{CO}_2$ carried by hemoglobin...	72.0		78.8		80.7	
	pH		pH		pH	
Arterial blood (pH values recalculated).....	7.296		7.310		7.281	
Venous blood (pH values recalculated).....	7.283		7.280		7.244	
Difference.....	0.013		0.030		0.037	

which it is unable to dialyze. This is peculiarly important for the higher organisms as they now exist because when in solution in the plasma it is quickly eliminated or destroyed. It disturbs the acid-base balance of a solution on account of its acidic properties. Blood after hemolysis is more acid than before (Milroy, 1917; Fridericia, 1920; Campbell and Poulton, 1920-21; Conway and Stephen, 1922), and while its potential  $\text{CO}_2$  carrying power is not altered, it can carry less  $\text{CO}_2$  at a particular pH. Conway and Stephen (1922) find that within

the physiological limits of  $\text{CO}_2$  tension the pH inside the corpuscle is 0.13 lower than in the plasma (i.e., the corpuscles are 35 per cent more acid than the plasma). If hemoglobin were present dissolved in plasma there would undoubtedly be little possibility of varying the concentration owing to disturbances in the osmotic relations which would doubtless occur. As it is, the concentration of hemoglobin may be increased and decreased without any osmotic disturbances merely through an increase or decrease of the number of red cells per unit volume of blood.

Hemoglobin is inclosed within a cell membrane which is freely permeable to water and  $\text{CO}_2$ , but the red cells do not carry all of the  $\text{CO}_2$  which the above discussion indicates is carried by base furnished by hemoglobin. A peculiar change is found to take place which has only recently been adequately explained and the physiological import is far from being completely appreciated. Schmidt (1867) and Zuntz (1867) independently discovered that serum from blood equilibrated with a high tension of  $\text{CO}_2$  contained more titratable alkali than serum from the same blood equilibrated at a low tension of  $\text{CO}_2$ . Zuntz erroneously concluded that sodium was split off from the cell proteins and diffused out from the corpuscles into the serum under the influence of increasing  $\text{CO}_2$  tensions and formed  $\text{NaHCO}_3$  in the serum. H. Nasse (1878) found that the chlorine in serum was decreased when blood was treated with high tensions of  $\text{CO}_2$ . The work was confirmed by Gürber (1895), Hamburger (1902) and others, who also demonstrated that there was no transfer of cations and that other anions also diffused to some extent. There were, however, some observations indicating a transfer of cations which have been refuted by the recent work of Doisy and Eaton (1921) and of Mukai (1921). Doisy and Eaton determined that the source of confusion had been due to failure to consider the loss of water from the plasma occurring when blood is brought into equilibrium with an increased  $\text{CO}_2$  tension. Taking this into account, they found by actual analysis that there was no transfer of sodium or potassium between plasma and corpuscles. Mukai found that the total amount of ash in serum remains constant if allowance is made for loss or gain of water, indicating that there is no exchange of cations.

The concentration of hemoglobin in the corpuscle is about 30 per cent, while the concentration of proteins in the serum is about 8 per cent. When alkali is furnished by protein owing to an increased  $\text{CO}_2$  tension (increased cH) much more alkali is furnished by the protein solution

in corpuscles. This tends to cause a much more rapid increase of  $\text{NaHCO}_3$  and less rapid increase of  $\text{cH}$  inside the cell than in the plasma.

But what actually occurs is that there diffuses into the corpuscles not only  $\text{H}_2\text{CO}_3$  (or  $\text{CO}_2$ ) to react with the cell proteins and other buffers, but also  $\text{HCl}$  formed from the  $\text{NaCl}$  of the plasma. The loss of  $\text{HCl}$  permits the plasma bicarbonate to increase more rapidly than would be the case if only the plasma buffers were reacting. The loss of  $\text{Cl}$  from the plasma accounts for about three-fourths of the gain of plasma bicarbonate (Van Slyke and Cullen, 1917; Fridericia, 1920; L. J. Henderson, 1921; Warburg, 1922). Doisy, Eaton and Chouke (1922) calculate that, of the total base furnished to form plasma bicarbonate when blood absorbs  $\text{CO}_2$  in changing reaction from pH 7.45 to 7.25, 16 per cent is furnished by the non-migrating serum buffers (plasma proteins, amino acids and organic acids). Of the remaining 84 per cent, 80 per cent is furnished by the migration of  $\text{Cl}$  into the corpuscles and the other 4 per cent probably by the migration of other acid radicles such as  $\text{SO}_4$  (deBoer, 1917) and  $\text{PO}_4$  (Doisy and Eaton, 1921). Doisy and Beckmann (1922) demonstrated that the  $\text{Cl}$  shift occurs in vivo by finding a change between arterial and venous blood. The transfer of  $\text{Cl}$  is such that about half of the added  $\text{CO}_2$  is carried in the plasma and half in the cells. The actual reactions which take place in blood when  $\text{CO}_2$  and  $\text{O}_2$  are absorbed or given off is shown in the following chart taken from an article by Austin, Cullen, Hastings, McLean, Peters and Van Slyke (1922).

An explanation for the cause of the transfer of chloride between corpuscles and plasma has been found in applying a consideration of Donnan's theory of membrane equilibrium to the simpler phases of the problem. (Warburg, 1922; Barcroft, Bock, A. V. Hill, Parsons, Parsons, and Shoji, 1922; Van Slyke, Wu and McLean, 1923.) Donnan proposed in 1911 an explanation for the unequal distribution of diffusible ions on the two sides of a membrane which is impermeable to one ion in solution. Take for example, two solutions, one containing  $\text{Na}$  ions and  $\text{Cl}$  ions and the other  $\text{H}$  ions and  $\text{Cl}$  ions, separated by a membrane impermeable to the  $\text{Na}$  ions but through which  $\text{H}$  and  $\text{Cl}$  ions can pass freely. This may be represented by the following scheme.



PLASMA	RED CELL WALL	CELL
(1) $\text{H}_2\text{CO}_3 + \text{Na Protein} \rightleftharpoons \text{H Protein} + \text{NaHCO}_3$		
(2) $\text{H}_2\text{CO}_3 + \text{NaCl} \rightleftharpoons \text{NaHCO}_3 + \text{HCl}$	$\xrightarrow{\text{HCl}}$	(3) $\text{HCl} + \text{K}_2\text{HPO}_4 \rightleftharpoons \text{KH}_2\text{PO}_4 + \text{KCl}$
		(4) $2\text{HCl} + 2\text{KHbO}_2 \rightleftharpoons 2\text{KCl} + \left\{ \begin{array}{l} \text{HHbO}_2 \\ \text{HHb} + \text{O}_2 \end{array} \right.$
	$\xrightarrow{\text{H}_2\text{CO}_3}$	(5) $\text{H}_2\text{CO}_3 + \text{K}_2\text{HPO}_4 \rightleftharpoons \text{KHCO}_3 + \text{KH}_2\text{PO}_4$
$\text{H}_2\text{CO}_3$		(6) $2\text{H}_2\text{CO}_3 + 2\text{KHbO}_2 \rightleftharpoons 2\text{KHCO}_3 + \left\{ \begin{array}{l} \text{HHbO}_2 \\ \text{HHb} + \text{O}_2 \end{array} \right.$
	$\xrightarrow{\text{O}_2}$	
$\text{O}_2$		

There will be a tendency for the H ions and Cl ions to distribute themselves uniformly on each side of the membrane. Let us assume that at the beginning the Cl ions were in equal concentration in both solutions. Some H ions will diffuse from (2) into (1) but they can only diffuse along with an equivalent quantity of Cl ions. This leads to an excess of Cl ions in (1) and a tendency for them to diffuse back into (2). When equilibrium is reached it is found that some H and Cl ions have diffused from (2) into (1) and the concentration of Cl ions is greater in (1) while the concentration of H ions is greater in (2). Donnan has proved that the distribution is such that

$$(H)_1 \times (Cl)_1 = (H)_2 \times (Cl)_2$$

The larger the concentration of the non-diffusible ion Na, the greater will be the inequalities in H and Cl ion concentration on the two sides of the membrane. Donnan and Harris' experiments (1911) indicate that if the ratio of NaCl to HCl is 100 to 1 at the beginning, only about 1 per cent of the HCl will diffuse from (2) to (1). This theory undoubtedly has wide applications in biology, as Donnan has suggested, and should prove very fruitful in studying the unequal distribution of ions which occurs frequently in many biological tissues.

If we assume that the semi-permeable membrane is the membrane of the red blood corpuscle and that (1) represents the blood plasma and (2) the fluid within the corpuscles we have a very simplified picture of the equilibria in the blood and one which shows why the red cells can be more acid than the plasma which surrounds them. The actual conditions are far more complicated. There are similar equilibria between bicarbonates, sulphates and inorganic phosphates inside and outside of the corpuscle. Neither the basic cations (sodium, potassium, magnesium and calcium), the proteins nor some organic phosphorus compounds<sup>3</sup> can diffuse through the cell membrane. As the sulphates and diffusible phosphates are present in very low concentration we may, for simplicity, consider the diffusible ions as being only H, Cl and  $HCO_3$ . From Donnan's law of membrane equilibrium there will be a distribution of the ions on the two sides of the corpuscular membrane so that:<sup>4</sup>

<sup>3</sup> Bloor (1918) has shown that there are 200 mgm. of combined phosphoric acid per 100 cc. of corpuscles.

<sup>4</sup> The subletter indicates concentration in the corpuscles C or in the serum S. This discussion is modified from Barcroft et al. (1922).

$$\begin{aligned}(\text{H})_C \times (\text{Cl})_C &= (\text{H})_S \times (\text{Cl})_S \\ (\text{H})_C \times (\text{HCO}_3)_C &= (\text{H})_S \times (\text{HCO}_3)_S\end{aligned}$$

Therefore:

$$\frac{(\text{H})_C}{(\text{H})_S} = \frac{(\text{Cl})_S}{(\text{Cl})_C} = \frac{(\text{HCO}_3)_S}{(\text{HCO}_3)_C}$$

and

$$\frac{(\text{H})_C}{(\text{H})_S} = \frac{(\text{Cl})_S + (\text{HCO}_3)_S}{(\text{Cl})_C + (\text{HCO}_3)_C}$$

As the (H) and serum protein concentration are very small (letting (B) represent concentration of total basic ions)

$$\begin{aligned}(\text{B})_S &= (\text{Cl})_S + (\text{HCO}_3)_S \\ (\text{B})_C &= (\text{Cl})_C + (\text{HCO}_3)_C + (\text{Hb})_C + (\text{PO}_4)_C \\ \frac{(\text{H})_C}{(\text{H})_S} &= \frac{(\text{B})_S}{(\text{B})_C - (\text{Hb})_C - (\text{PO}_4)_C}\end{aligned}$$

Van Slyke, Wu and McLean have recently shown that these as well as other relations hold true only when the concentrations are calculated in terms of gram equivalents of solute per grams of water as used in osmotic pressure calculations rather than in terms of gram equivalents of solute per volume of solution as customarily employed. As the water concentration in plasma is about 90 per cent and that of corpuscles 70 per cent the differences resulting from the two methods of calculation are considerable. Although the total concentration of base in the corpuscles (calculated on the water basis) is greater than in serum, the concentration of base actively concerned in the Donnan equilibrium is less owing to the large proportion which is combined with (balanced by) hemoglobin (and organic phosphate) and, therefore, has no influence on the equilibrium. According to the above equation, if the concentration of effective base is less in corpuscles, the cH of the corpuscles must be greater than the cH of the plasma? This conclusion based on Donnan's theory of membrane equilibrium is substantiated by many experiments (Milroy, 1917; Parsons, 1917; Fridericia, 1920; Joffe and Poulton, 1920-21; Campbell and Poulton, 1920-21; Evans, 1921b; Conway and Stephen, 1922) which demonstrate that the reaction in the corpuscle is actually more acid than the plasma. Warburg (1922) reports, that at pH 7.4, the pH of horse corpuscles is 0.09 to 0.13 less

than plasma and Conway and Stephen (1922) have shown with human blood that laked corpuscles have a pH 0.13 lower than plasma.

There is a tendency for each ion to distribute itself equally between solutions inside and outside of the corpuscle which is counteracted by the attractive forces of the non-diffusible ions, with a resultant unequal distribution when equilibrium is finally reached. When blood is brought in contact with a higher tension of  $\text{CO}_2$ , the proteins of the corpuscles furnish more base than do the proteins of the plasma so that the increase in  $(\text{HCO}_3)$  will be greater in corpuscles than in plasma. As  $(\text{HCO}_3)$  increases more rapidly in corpuscles the  $(\text{H})$  within the corpuscles will rise less rapidly than in the plasma.

But with the unequal increase in  $(\text{HCO}_3)$  and  $(\text{H})$  there is a disturbance of the ion equilibria and a shifting of the ions results. Some  $\text{HCO}_3$  ions diffuse out of the corpuscles and about an equal number of  $\text{Cl}$  ions diffuse in. Other diffusible ions in the plasma ( $\text{PO}_4$  and  $\text{SO}_4$ ) take part in the interchange so that the rise of  $(\text{HCO}_3)$  in the plasma can be almost completely accounted for by loss of other ions from the plasma. Diffusion takes place until the increase of  $\text{HCO}_3$  is distributed practically equally between corpuscles and plasma. The cause of the shifting of all the diffusible ions when only the equilibrium of  $(\text{HCO}_3)$  has been disturbed is, as stated by Warburg, in accordance with Donnan's theory and due to the tendency for the new equilibria to be established by a shifting which causes the same relative change in concentration (activity) of all diffusible ions.

These mechanisms are of importance in a consideration of the physiology of neutrality regulation. The corpuscles act as a storehouse for base which is furnished when oxygen is lost or carbon dioxide absorbed by the blood. By this mechanism the corpuscles neutralize most of the carbonic acid which enters the blood and, as a result, the acidity of the plasma increases much less rapidly than it otherwise would. Separated serum (serum with no corpuscles present) changes in reaction with changes of  $\text{CO}_2$  tension much more rapidly than true serum (serum in contact with corpuscles) (Joffe and Poulton, 1920-21; Evans, 1921b).

The storage or release of  $\text{H}^+$  ions from the corpuscles with increase or decrease in  $\text{CO}_2$  tension provides a much more delicate adjustment of reaction in the plasma and large changes in  $\text{CO}_2$  tension can cause only small changes in its pH. With a limited change in pH of plasma the variation in  $\text{CO}_2$  tension possible will depend on the amount of  $\text{H}^+$  ions which can be taken up. This will depend mainly on the relative quantity of corpuscles available and, of course, on the amount of the

buffers in each corpuscle. The latter variation, however, seems to be slight. The increased amount of buffer available as the percentage of corpuscles increases produces a steep  $\text{CO}_2$  absorption curve: relatively smaller amounts of  $\text{CO}_2$  absorbed at low  $\text{CO}_2$  tensions and relatively larger amounts absorbed at high tensions. In the body, therefore, the  $\text{CO}_2$  interchange can occur with smaller changes in reaction of the blood, or conversely, a greater  $\text{CO}_2$  interchange is possible within the normal limits of blood reaction. The other extreme is evident when the quantity of corpuscles is low in a unit volume of blood. The  $\text{CO}_2$  absorption curve approaches that of separated serum, so that the  $\text{CO}_2$  interchange in the body causes greater changes in  $\text{cH}$  of the blood.

EXCHANGE OF WATER BETWEEN CORPUSCLES AND PLASMA. H. Nasse (1878) observed in experiments on the  $\text{CO}_2$ -carrying power of blood, that the corpuscles swelled when treated with increasing tensions of  $\text{CO}_2$ . This phenomenon was studied repeatedly but no adequate explanation was available until Spiro and Henderson (1909) proposed the explanation embodied in the following discussion.

With the change in reaction due to the increase or decrease in carbonic acid in the blood there are changes in the volume of the red cells brought about by alteration in the osmotic pressures of the fluids inside and out. When carbonic acid is added to blood the  $\text{cH}$  is increased and base is liberated from the protein salt to form ionized bicarbonate and unionized protein. The addition of a molecule of carbonic acid, therefore, causes the formation of three osmotically active particles in place of two. This reaction takes place to a greater extent in the corpuscles than in the plasma, as described above. The osmotic pressure of the corpuscles will, therefore, increase more rapidly than that of the plasma. As the corpuscles are elastic and permeable to water, water will enter and cause the corpuscles to swell until the osmotic pressures inside and out are the same. This reaction will take place regardless of the  $\text{Cl}$  shift which apparently has nothing to do with the total osmotic changes. Van Slyke, Wu and McLean (1923) have found that the water interchange can be explained quantitatively only when the concentrations of osmotically active particles are calculated in terms of gram equivalents per grams of water.

The change in volume of the cells causes an increase or decrease in water content of the surrounding plasma. The loss or gain of water by the plasma is not accompanied by loss or gain of salts (Doisy and Eaton, 1921; Mukai, 1921; Warburg, 1922) so their concentration changes with change in volume of the cells. These alterations in concentration have

been neglected until recently and have changed substantially some conclusions based on analyses of plasma.

The increase or decrease in volume is not great. Doisy and Eaton found in beef blood outside the body that the corpuscles occupied 43 volumes per cent in an atmosphere of 3 per cent  $\text{CO}_2$  and 46 volumes per cent in pure  $\text{CO}_2$ . These authors studied the change in cell volume between arterial and venous blood and found wide variations with an average increase in venous blood of 0.35 volume per cent. It should be emphasized, however, that the changes taking place within the body are much more complicated than in a test tube and the regulation of the fluid interchange between blood and tissues cannot be explained so simply.

**THE EQUILIBRIA IN THE BLOOD.** In the normal interchange of gases in lungs and tissues all of the oxygen is not given up in the tissues nor all the carbon dioxide in the lungs. The large excess remaining in the blood at all times increases the flexibility of the regulating mechanisms and furnishes a great factor of safety for periods of unusual activity on the part of the organism.

The following table shows the distribution of the constituents which we have discussed above, in normal human blood under resting conditions. The concentrations are calculated on the volume basis.

ARTERIAL BLOOD	VENOUS BLOOD
(1) pH 7.30-7.40	7.27-7.37
(2) Free $\text{CO}_2$ 1.5-4.0 vol. per cent (0.67-1.8 mM)	1.7-4.2 vol. per cent (0.76-1.87 mM)
$\text{CO}_2$ tension 22-63 mm. Hg.	25-65 mm. Hg.
(3) Combined $\text{CO}_2$ 31-56 vol. per cent (14-25 mM)	36-61 vol. per cent (16-27 mM)
(4) Plasma Cl 370-390 mgm. per 100 cc. (105-110 mM)	360-370 mgm. per 100 cc. (102-107 mM)
(5) Percentage saturation of Hb 93-98	62-85
(6) $\text{O}_2$ tension 84-100 mm. Hg.	30-60 mm. Hg.
$\text{O}_2$ content 17-22 vol. per cent (7.6-9.8 mM)	11-16 vol. per cent (4.9-7.1 mM)

Under normal resting conditions these values show little tendency to change. The extreme constancy is brought about by the delicate regulation of the aeration of the blood in the lungs. It is important to note that, as L. J. Henderson (1921) has emphasized, a change in any one of the six different variables listed above causes a change in all or all but one of the others. The work of Haldane (1922) has demonstrated

that the variations in oxygen and carbon dioxide are the factors of fundamental importance to the organism and that, of these, the regulating mechanisms are mainly concerned with the carbon dioxide elimination.

**THE STIMULUS OF THE RESPIRATORY CENTER.** The ventilation of the lungs is regulated by the respiratory center in the medulla. Rhythmic expansion and contraction of the lungs is brought about by nerve impulses from the center to the muscles concerned with respiratory movements. The movements are adjusted in rate and depth so that the blood becomes sufficiently aerated. As the activity of the center and the resultant breathing are regulated by the blood which passes through it, it is important to determine what property of blood calls the center into action.

The specific substance which exercises control over the chemical regulation of respiration has been the subject of great discussion and frequent experimentation. Haldane and Priestley (1905) demonstrated conclusively that variations in the alveolar  $\text{CO}_2$  tension called forth proportional responses of the respiratory center and consequent ventilation of the lungs and concluded that in this way the  $\text{CO}_2$  tension of the respiratory center controlled the arterial  $\text{CO}_2$  tension. The experiments of Winterstein (1911, 1915) and Hasselbalch (1912) on animals seemed to prove that the hydrogen ion concentration of the blood is the effective stimulus rather than the  $\text{CO}_2$  tension. Laqueur and Verzář (1911), however, believed that the carbonic acid itself is a specific stimulus to the respiratory center rather than the H ions which the acid might produce. Since that time physiologists have been divided as to the actual agent which produces the stimulation, though the general view has been that the H ions are the effective stimulus.

Haggard and Y. Henderson (1919) have suggested another factor, "respiratory X" as an additional stimulus. Winterstein (1921) recently modified his theory proposing two ways in which the  $\text{cH}$  of the center may be influenced: first, by an increase or decrease in  $\text{cH}$  of the blood, and second, by the formation of acids in the center itself. The second postulate explains the response of the center in conditions of oxygen want as due to the formation of increased quantities of acid metabolic products in the center resulting in an increased  $\text{cH}$ .\*

The problem of the specific stimulus has not been an easy one to solve. If the stimulation has something to do with the  $\text{CO}_2$  tension of the solution, we must consider the possible action of  $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$ , H ions and

\* See also Gesell (1923).

$\text{HCO}_3$  ions (we cannot, at present distinguish between dissolved  $\text{CO}_2$  and  $\text{H}_2\text{CO}_3$  so they may be considered together). Changes in  $\text{CO}_2$  tension are, therefore, usually accompanied by similar changes in  $\text{cH}$ . It is possible, however, to increase the  $\text{CO}_2$  tension and at the same time decrease the  $\text{cH}$  but only by introducing other complicating factors such as  $\text{Na}$  ions and  $\text{HCO}_3$  ions into the solutions. These other ions may stimulate the center or may change its irritability to other stimuli.

The following brief summary of evidence from recent experimentation may illustrate the difficulties attending this work. Hooker, Wilson and Connet (1917) showed that when the medulla of a dog was perfused with blood having a  $\text{cH}$  maintained by  $\text{CO}_2$ , the response of the respiratory center was much greater than when the perfusion was carried out with blood in which the  $\text{cH}$  was made the same by adding  $\text{HCl}$ . In these experiments, though the  $\text{cH}$  and  $\text{Na}$  ion concentration were kept constant, the blood which caused the greater stimulation contained higher concentrations of  $\text{H}_2\text{CO}_3$  ( $+\text{CO}_2$ ) and  $\text{HCO}_3$ . Scott's (1918-19) experiments, demonstrated that the respiratory center reacted similarly to increasing  $\text{CO}_2$  tensions whether the reaction of the blood was normal or more alkaline than normal. In these experiments it would appear that the respiratory center responded to changes in  $\text{H}_2\text{CO}_3$  ( $+\text{CO}_2$ ) or  $\text{HCO}_3$  regardless of the reaction of the blood. When breathing atmospheric air the ventilation was practically the same in each experiment, though the  $\text{HCO}_3$  was higher in one than in the other. Collip (1920-21) showed that slow injections of  $\text{NaHCO}_3$  caused increased breathing. Here it may be assumed that increased breathing was accompanied by an increase in  $\text{Na}$ ,  $\text{HCO}_3$  and  $\text{H}_2\text{CO}_3$  and a decrease in  $\text{cH}$  of the blood. Dale and Evans (1922) injected  $\text{NaHCO}_3$  until the reaction of the blood was  $\text{pH}$  8.0 without producing apnea. As in the experiments of Collip, the low  $\text{cH}$  was accompanied by increased  $\text{Na}$ ,  $\text{HCO}_3$  and  $\text{H}_2\text{CO}_3$ . In both of these investigations the  $\text{H}_2\text{CO}_3$  seemed to be less effective in the presence of high  $\text{HCO}_3$  and low  $\text{cH}$ .

The experiments outlined above all appear to point to the conclusion that an increase in  $\text{H}_2\text{CO}_3$  or  $\text{HCO}_3$  stimulates the respiratory center and that it is relatively insensitive to variations in  $\text{cH}$  as such. It should be noted that all of these studies were made on anesthetized or decerebrate animals, and on the alkaline side of the normal reaction of the blood.

Seemingly contradictory evidence has been obtained by studies on men with apparently normal respiratory centers. In ordinary types of "acidosis" the blood may contain much less than the normal concen-

tration of  $\text{H}_2\text{CO}_3$  and  $\text{HCO}_3$  with the cH and respiration both probably slightly increased. On the other hand, after bicarbonate feeding, the blood is high in  $\text{H}_2\text{CO}_3$  and  $\text{HCO}_3$  while the respiration is depressed and the cH probably lowered somewhat. The experiments of Haggard and Y. Henderson (1919) are in agreement with the above. After forced breathing, apnea results with low  $\text{H}_2\text{CO}_3$ , low  $\text{HCO}_3$  and low cH.

From the available data it seems apparent that both cH and the concentration of  $\text{H}_2\text{CO}_3$  (or  $\text{HCO}_3$ ) may play a part in the stimulus of the respiratory center. In attempting to correlate the seemingly conflicting evidence, the lack of available data on the permeability of the membranes between the blood plasma and the respiratory center renders an intelligent discussion difficult. Jacobs (1920, a, b) has shown that  $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$  has great power of penetration through animal fluids and membranes. He has suggested that the stimulation of the respiratory center is due not only to the hydrogen ions from the blood acting at the cell surface but also to the action of the rapidly penetrating  $\text{CO}_2$  in changing the cH within the center itself.

One may postulate that the equilibrium existing between the plasma and respiratory center may be somewhat analogous to the equilibrium existing between corpuscles and plasma, though we do not know at present what differences in permeability may exist in the two systems. If we assume an unequal distribution of bicarbonate and H ions between plasma and center and a mechanism similar to that found in blood, i.e., with increased alkalinity the difference in cH between center and plasma is increased, the following relations would occur. As bicarbonate is increased in the plasma the difference between center and plasma is increased. As  $\text{H}_2\text{CO}_3$  increases to restore the cH and distributes itself proportionately between center and plasma the cH in the center would increase more rapidly due to the relatively lower  $\text{HCO}_3$ . The increased cH of the center would stimulate respiration increasing the elimination of  $\text{CO}_2$  until the cH of the center was restored to normal, at which time the plasma would be more alkaline than normal. With low bicarbonate in the plasma the difference between plasma and center will be less and a normal cH of the center will be maintained when the cH of the plasma is greater than normal. With these assumptions all of the experiments are in agreement though the relations are, of course, far from being explained quantitatively.

The threshold of stimulation of the respiratory center by materials from the blood is definitely altered under certain conditions. When the center receives an insufficient amount of oxygen, respiration is greater

with the same concentration of  $\text{CO}_2$  or  $\text{H}^+$  ions; the center is said to be more irritable. Whether or not this is due to change in concentration of  $\text{H}^+$  ions, in the center itself, as suggested by Winterstein, has not been demonstrated. Decreased irritability is indicated after morphine injections when a greater  $\text{cH}$  is required to stimulate normal respiration. No single explanation is available to cover all conditions which are now described as due to change in irritability of the center though one may imagine that changes in irritability may be in some instances merely expressions of physico-chemical changes in the equilibrium existing between the blood plasma and fluids in the center.

Although the specific stimulus of the respiratory center is chemical, there is also a nervous mechanism regulating respiration which normally comes into play. When the lungs are distended certain sensory nerve endings of the vagus in the lung tissue are stimulated, causing a reflex inhibition of inspiration and onset of expiration. Similarly the collapse of the lungs stimulates other nerve endings bringing about a new inspiration. Thus the rate of respiration is increased above the rate found when the vagi are cut. This nervous regulation of respiration in conjunction with the chemical regulation makes possible very fine adjustments to the needs of the body. When greater ventilation is needed, due, for instance, to increased  $\text{cH}$  in the blood, it can be most effectively accomplished by an increase in both rate and depth of respiration. The respiratory center, under these circumstances, sends out stronger impulses than usual, increasing the depth of respiration, but tending to cause a decrease in rate. The rate is maintained or even augmented by the reflex action of the vagus.

**REGULATION OF CIRCULATION.** The importance of the circulation in a study of the acid-base balance in the body is evident, since it is by means of the blood stream that the acid products of metabolism are transported to the lungs and kidneys. Changes in the arterial blood pressure have been studied in attempting to obtain some information concerning the factors influencing the transportation of these materials. Diminished blood pressure and circulatory failure following excessive artificial respiration have been studied by Y. Henderson (1908) and by Y. Henderson and Haggard (1918) and have been attributed to the loss of carbonic acid. More recently the view has been held that the lowering in blood pressure is due to diminished  $\text{cH}$  of the blood (Y. Henderson, and Haggard, 1920; Haldane, 1922). The recent work of Dale and Evans (1922) demonstrates conclusively that the effect obtained by these investigators must have been due, not to the change

in cH of the blood, but directly to the decrease in  $\text{H}_2\text{CO}_3$ , independent of the cH of the blood. Dale and Evans showed that a change of pH in arterial blood to 8.0 by injecting sodium bicarbonate did not produce any change in arterial pressure, while the removal of  $\text{H}_2\text{CO}_3$  by excessive ventilation with a similar change of pH did cause an extreme fall in arterial pressure. The pH of both arterial and venous blood changes comparably by these procedures. Their experiments show that the fall of arterial pressure which accompanies excessive artificial ventilation is due to the depression of the vasomotor centers of the bulb and spinal cord by a decrease in the concentration of  $\text{H}_2\text{CO}_3$  in the blood and not due to the change in cH. If we attempt to apply these conclusions to the smaller variations which may occur in the more normal individual, a complication is encountered similar to that found in the case of the respiratory center, i.e., there seem to be no constant differences in blood pressure between individuals with high and low concentrations of  $\text{H}_2\text{CO}_3$  in the blood. Other factors seem to overshadow any effects which might be due to these causes.

**ELIMINATION OF ACIDS AND BASES BY THE KIDNEY.** The influence of urine excretion on the regulation of neutrality in the organism has been analyzed in great detail by L. J. Henderson (1909, 1911) and Henderson and Palmer (1912-15) in a long series of investigations. Their careful analysis of the factors concerned with acid excretion gives us a clear understanding of the fundamentals of this important regulating mechanism.

Practically all of the food materials absorbed from the gastro-intestinal tract must be eliminated in one form or another by way of the lungs or kidneys. In man most of the carbonic acid formed in the body is lost through the lungs. Much of the water is eliminated by way of the lungs and skin. Most of the inorganic salts and organic matter (not  $\text{CO}_2$ ) is excreted by the kidneys together with a considerable amount of water. The kidneys are therefore called upon to regulate not only the osmotic relations within the organism, but also the relative quantities of basic and non-volatile acidic radicles. All of the basic radicles and part of the acidic radicles are taken in as salts in the food while some of the acids, such as sulphuric and phosphoric, are formed from neutral protein material by oxidation in the organism. Some foods contain salts (such as acid potassium citrate) of acids which are oxidized to carbonic acid and thus furnish base to the body (Sherman and Gettler, 1912). The normal food of man contains less basic radicles (such as sodium, potassium, calcium and magnesium) than acidic radicals, if one includes the

phosphorus and sulphur of the proteins which in the body are oxidized to phosphoric and sulphuric acids. In pathological conditions such as diabetes mellitus, large quantities of organic acids may be formed and must be eliminated. Occasionally, to a less extent, due to changes in dietaries or unusual conditions of stress, bases tend to accumulate in the organism. The presence of an effective mechanism for eliminating an excess of bases or acids from the body is therefore necessary. As an excess of acid in the body is more liable to occur, the mechanism for eliminating acid and conserving base is of the greatest practical interest.

Normal human urine ranges in reaction from pH 4.8 to 7.4 (Henderson and Palmer, 1912, 1914; Newburg, Palmer and Henderson, 1913). At these reactions the strong acids, such as sulphuric and phosphoric, cannot be eliminated as such but only in the form of salts. At pH 4.8, 1 mol of sulphuric acid is combined with nearly two mols of base. One mol of phosphoric acid is combined with a little less than 1 mol of base ( $\text{NaH}_2\text{PO}_4$ ), whereas in the body where the reaction is more alkaline, the phosphoric acid is combined with 1.8 mols of base. It is evident, then, that in the excretion of 1 mol of phosphoric acid, more than 0.8 mol of base may be retained by the body and serve to neutralize other molecules of acid. If, however, there is an excess of base in the body, it may be excreted as disodium phosphate or bicarbonate and a decrease in the pH of the urine will result.

This limit of reaction of the urine must necessarily limit the efficiency of the kidneys for excreting acids and might easily lead to an excessive loss of base or a piling up of acid in the organism. However, the substitution of ammonia for some of the fixed base occurs and permits the elimination of acids in the form of neutral salts, and a retention of the fixed base by the body. Each mol of ammonia thus represents the saving of one mol of base for the body.

Ammonium ions when present in the blood are toxic so that the normal concentration there is extremely low. It has been taught for years that this small quantity in the blood is maintained by a small portion of the ammonia (produced during the process of deamination of amino acids) which had not been changed into urea and furnishes ammonia for neutralization of acids. The urinary ammonia has frequently been found to increase at the expense of the urea and the process of urea formation from ammonia seems to be reversible (Barnett and Addis, 1917).

Nash and Benedict (1921, 1922) have recently shown that the ammonia which appears in the urine is formed by the kidneys and not by

the liver. This observation furnishes a much more satisfactory basis for explaining the delicately adjusted mechanism for excreting bases and acids which regulates the acid-base equilibrium of the body. With the formation of ammonia localized in the kidney, the retention of base by substitution of ammonia, and the retention of base during the excretion of acid radicles by variations in  $\text{cH}$  of the urine may be more closely related. Decrease in the alkali reserves of the body may be explained as due to 1, inability of the kidney to excrete acids as rapidly as they are formed by eliminating them as such, or as ammonium salts; 2, the inability of the kidney to excrete acid radicles even though the mechanism for ammonia formation is unimpaired; 3, the loss of power to form ammonia with the resulting loss of fixed alkali. The necessity for the excretion of an excess of basic radicles occurs less frequently on account of the limits placed by the gastro-intestinal tract, by vomiting and diarrhea, on the introduction of large quantities of salts, and the absence of formation of base (even organic) in the body.

Studies of the influence of acids and alkalis on the excretion of ammonia date back to the classic work of Walter (1877) who found increased excretion of ammonia in dogs after injection of hydrochloric acid. (It is interesting to note in passing that rabbits do not respond in the same way as they have practically no mechanism for forming ammonia.) Marriott and Howland (1918) found that the ingestion of  $\text{HCl}$  by man gave an increase in ammonia excretion, while large quantities of  $\text{NaH}_2\text{PO}_4$  did not. The ingestion of  $\text{NaHCO}_3$  causes the ammonia excretion to diminish (Janney, 1911-12) and after large doses it disappears completely from the urine (Davies, Haldane and Kennaway, 1920).

The reaction of the urine, while an index of the output of acids, does not furnish much evidence concerning the quantity of acid excreted. Such information may be obtained by titration. Many methods of titrating have been used, the most popular being that in which phenolphthalein (end point,  $\text{pH}$  8.5) is used as the indicator. L. J. Henderson (1911) emphasized that its use was due mainly to its convenience and suggested as a more scientific procedure, titration to the  $\text{pH}$  of the blood. Such a titration yields an estimate of the amount of acid excreted over and above the normal reaction of the blood, i.e., the physiological elimination of acids. On account of the buffering of the phosphates, the difference between the two methods of titration may be considerable. As it has been shown that the ammonia of the urine represents acid eliminated from the body, the equivalent amounts of

ammonia and titratable acid may be added together to furnish information concerning the total acid excreted. There is, however, a considerable amount of ammonia in urine, even at pH 7.4 (the normal reaction of the blood) and it disappears only when the reaction becomes more alkaline. Fitz and Van Slyke (1917) suggested, for this reason, that the older method of titrating with phenolphthalein is more satisfactory in that, as the end point more nearly approaches the reaction of the urine obtained when the ammonia excretion is zero, the ratio of ammonia to titratable acid remains more nearly constant. For obtaining an estimate concerning the relative sensitiveness of the two kidney mechanisms, i.e., base retention by ammonia formation, and baseretention by alteration in the ratio of basic to acid phosphate, the titration with phenolphthalein would seem to be the more satisfactory.

Under normal conditions, an excess of acid must be excreted by the human kidney if the store of base is to be maintained. This is accomplished by the excretion of urine more acid than the blood and containing a greater ratio of  $\text{NaH}_2\text{PO}_4$ :  $\text{Na}_2\text{HPO}_4$  than existed in the blood, thereby retaining a portion of the base. Part of the acidic radicles are eliminated combined with ammonia formed in the kidney, with a further retention of fixed base. Gamble (1922) has recently studied the rôle of these mechanisms in retaining base. The three variables,  $\text{cH}$ , acid titration and ammonia, tend to rise and fall together. As most of the titratable acidity is due to phosphates, it may be said that the kidney makes use of two mechanisms for eliminating acids; the excretion of  $\text{H}_2\text{PO}_4$  and the formation of ammonia in roughly equivalent amounts, with a greater saving of base to the body the more acid the urine becomes. Actually, the acid-forming mechanism seems to be more sensitive and elastic, as greater changes occur in the acid titration than in the ammonia excretion (L. J. Henderson and Palmer, 1914; Davies, Haldane and Kennaway, 1920).

When abnormal organic acids such as  $\beta$ -oxybutyric acid and acetoacetic acid are present, the urine may become sufficiently acid so that a considerable proportion of these acids is excreted uncombined with base. Under these conditions another chemical process takes place which is distinctly favorable to the organism. A considerable portion of the acetoacetic acid formed in the body is decomposed to form carbonic acid (which can be eliminated by the lungs) and the neutral compound acetone, which can be eliminated by the kidneys without loss of any base. It actually happens that a large proportion of the total acetoacetic acid which is formed in the body and cannot be burned is excreted in a form which requires no loss of base from the body.

Excessive quantities of base can be removed more easily. The limit seems to be merely that  $\text{NaHCO}_3$  cannot be excreted in a solution more concentrated than 0.3 N, so, with sufficient quantities of water available, large quantities of base may be eliminated. Most of the determinations of the cH of alkaline urines reported in the literature are grossly in error, owing to the loss of free carbonic acid. Marshall (1922) and Gamble (1922) found that even after bicarbonate ingestion the urine never became more alkaline than pH 8.0, the limiting factors apparently being the maximum concentration of bicarbonate in urine and the  $\text{CO}_2$  tension in the kidney tissue.

**THE REGULATION OF URINE ACIDITY.** Although the usual physiological discussion lays emphasis on the fact that the kidneys aid in regulating the acid-base balance of the blood, the problem of greatest chemical interest is just the reverse. How does the acid-base balance of the blood regulate urine excretion? What factors in the blood influence the elimination of each of the acidic and basic radicles from the body?

The problem is of even greater complexity than the similar problem in connection with the regulation of the action of the respiratory center. Whereas the respiratory center is concerned with the elimination and absorption of the gases of the blood, the kidney is concerned with the elimination of solid materials which must be excreted in solutions not too concentrated, leaving the blood with a suitable osmotic pressure. Not only must the total osmotic pressure of the body fluid be kept within normal limits but also the concentration of each constituent in the blood.

Most of the available information indicates that the cH of the urine varies with the cH of the blood. As the urine is normally more acid than the blood plasma, it is similar in this respect to the fluid in the corpuscles but with this striking contrast. Whereas the difference in reaction between corpuscles and plasma diminishes as the blood becomes more acid, until the two fluids have the same reaction at a pH of about 6.5, the difference in reaction between urine and blood plasma increases, reaching a maximum when the urine has a pH of 4.7 and the blood a pH not much less than 7.0. With increasing alkalinity, the two fluids have the same reaction in the neighborhood of pH 7.8.

In general, as the plasma becomes more acid, the cH, acid titration and ammonia in the urine increase. This is shown in experiments involving the administration of acids or morphine or breathing high tensions of  $\text{CO}_2$  (for reference, see below). The experiment may be reversed by administration of  $\text{NaHCO}_3$  or by forced breathing, when the

plasma becomes more alkaline and the  $\text{eH}$  of the urine, titratable acidity and ammonia decrease. There is, however, at least one interesting exception to this simple control. Several hours after a severe hemorrhage the blood becomes more alkaline than normal while there is an increased excretion of acid and ammonia in the urine, which may persist for several days (Evans, 1921, a; Endres, 1922, and unpublished observations of the author). Evans has described this as a favorable mechanism for maintaining a higher alkalinity in the blood, but such an explanation is hardly helpful in studying the physico-chemical mechanism underlying the observation.

Haldane and his co-workers (Davies et al., 1920, 1922; Baird et al., 1923) have reported observations showing a very interesting inter-relationship involved in the excretion of the two acidic radicals  $\text{Cl}$  and  $\text{HCO}_3$ . They have shown that the sum of the two cannot exceed  $0.3 \text{ N}$  in the urine and a diminution in the concentration of  $\text{HCO}_3$  in the blood is accompanied by a rise in  $\text{Cl}$  without any tendency toward increased excretion of  $\text{Cl}$  in the urine.

**VARIATIONS IN THE ACID-BASE EQUILIBRIUM.** In applying a consideration of the mechanisms involved in neutrality regulations in the body to the everyday life of the individual one is struck by the constant need for such mechanisms. The student of today, to whom the narrow limits of reaction compatible with life have been emphasized so frequently, is liable to exaggerate the narrowness of these limits. On examining the data available at present one is inclined to place the limits of acidity and alkalinity compatible with life at about  $\text{pH}$  6.9 to 9.0 and one is surprised to find that these limits are closely approached in some of the ordinary activities of life. It would appear that the factors of safety are not so extensive in this regard as in many functions in the body, but such a conclusion is hardly justified when one considers the numerous mechanisms which may be called into play, the summation of whose activities is more than sufficient to cope with the extremes of acid or alkali excess under normal conditions. Nevertheless the organism may be seriously embarrassed at times by exceeding the ability of these mechanisms to maintain optimum conditions. A brief survey of some of the alterations in the acid-base equilibrium, encountered in activities which may be classed as not unusual, may be of interest, even though much of the data is incomplete.

**DAILY VARIATIONS.** There is evidence of a slight but definite change in the irritability of the respiratory center at different times of the year as indicated by variations in the alveolar  $\text{CO}_2$  of as much as 2 to 5 mm.

Hg (Straub et al., 1914-15 a). Far more striking changes occur during the course of 24 hours. Numerous investigators (Straub et al., 1914-15 a; Leathes, 1919; Endres, 1922) have shown that the alveolar  $\text{CO}_2$  tension is greater at night than during the day. The high alveolar  $\text{CO}_2$  is associated with a lowered alkali reserve (Collip, 1920) and with the excretion of urine which compared with the day's urine contains more ammonia, a higher titratable acidity and has a higher cH (Leathes, 1919; Campbell and Webster, 1921, 1922 b; Endres, 1922). All of these observations indicate that the cH of the blood is higher at night than during the day due presumably to a decreased irritability of the respiratory center during sleep. Higgins (1914) found that even a change in posture, such as lying down, increased the alveolar  $\text{CO}_2$ .

EFFECT OF DRUGS AND CHANGES IN NERVOUS EXCITABILITY. Changes in irritability of the respiratory center obtained by the use of drugs produce alterations in the acid-base equilibrium of the body. Morphine, which depresses nervous sensibility, causes a rise of alveolar  $\text{CO}_2$  (Straub, et al., 1914-15 b; Endres, 1922) and excretion of urine with a higher cH. Caffein causes a lower alveolar  $\text{CO}_2$  and lower cH in the urine, and similar changes in alveolar  $\text{CO}_2$  have been observed in individuals who were excited and anxious. Increased or decreased nervous excitability, therefore, seems to be reflected in the respiratory center which responds accordingly and causes the aeration of the blood to be adjusted to a slightly different level of cH, which is maintained in part by the respiratory center and in part by the kidneys.

THE "ALKALINE TIDE" AFTER MEALS. During the day, other factors come into play to cause alterations in the acid-base equilibrium which may be of sufficiently short duration to escape notice or render observation difficult. A phenomenon which has been known for many years is the "alkaline tide" in the urine, frequently observed after meals. The cause of the alkaline urine has been assumed to be the relative excess of base in the blood caused by the loss of the hydrochloric acid secreted into the stomach during the course of digestion. The hypothesis and even the observations have been criticised (Hasselbaleh, 1912; Leathes, 1919) but the phenomenon has been observed so frequently that there seems no doubt about its occurrence (Campbell and Webster, 1921; Fiske, 1921; Endres, 1922 and Hubbard and Munford, 1922). The careful experiments of Dodds and his collaborators (1921-23) (also Bennet and Dodds, 1921) prove beyond a doubt that the secretion of HCl into the stomach leaves the body tissues with an excess of base, which results in a retention of  $\text{CO}_2$  and a rise in bicarbonate of the blood,

an increased alveolar  $\text{CO}_2$  (also Higgins, 1914; Straub et al., 1914-15 c; Endres, 1922) and a blood probably slightly more alkaline than normal. The kidneys respond to the decreased  $\text{cH}$  of the blood by excreting less acid and less ammonia. There is a rise of 2 to 6 mm. in alveolar  $\text{CO}_2$  within half or three-quarters of an hour after meals, the height varying with the amount of  $\text{HCl}$  secreted into the stomach. A subsequent fall occurs to 2 to 6 mm. below the normal level due to the pouring out of alkaline pancreatic juice. These observations were controlled by the introduction of food products directly into the stomach and into the duodenum, and with other experimental procedures.

**GASTRIC TETANY.** The effect of loss from the blood of  $\text{HCl}$  secreted in the gastric juice is brought out most strikingly in conditions of gastric obstruction when the gastric juice cannot pass into the duodenum but is vomited. In these cases, the continuous loss of  $\text{HCl}$  results in a rapid increase in the bicarbonate of the blood, and tetany, which is quickly fatal if the obstruction is complete (McCann, 1918; MacCallum, et al., 1920; Hastings, Murray and Murray, 1921).

**ADMINISTRATION OF BICARBONATE.** A similar though less extreme condition results from administration of sodium bicarbonate by mouth or by intravenous injection. There results an increased concentration of bicarbonate in the blood, increased alveolar  $\text{CO}_2$  tension, more alkaline blood and the typical response of the kidney: urine less acid and containing less ammonia.

**VOLUNTARY OVERVENTILATION.** In the condition of overventilation the picture is rather different (Grant and Goldman, 1920; Collip and Backus, 1920; Davies, Haldane, and Kennaway, 1920; Koehler, 1923). An excess of carbonic acid is driven off from the lungs and the  $\text{cH}$  of the blood lowered thereby (pH 7.45 changed to pH 7.65 in one experiment). The  $\text{CO}_2$  combining power of the blood is not appreciably altered in these experiments (Davies, Haldane and Kennaway, 1920) though, of course, the  $\text{CO}_2$  tension is diminished. The kidneys respond by excreting base, the acidity and ammonia excretion diminish and organic acids may appear.

Symptoms of tetany may result from each of the above conditions in which there is but one point in common, namely, blood more alkaline than normal. In reviewing the work on tetany, Greenwald (1922) pointed out that the excess alkalinity of the blood will result in diminishing the dissociation of oxyhemoglobin and thereby tend to induce an oxygen want in the tissues. The muscle tetany and the oxygen want *per se* may both cause the development of lactic acid which can act as

a protective agent in decreasing the alkalinity of the blood and thereby causing a greater dissociation of oxyhemoglobin.

**HIGH TEMPERATURES.** An increase in temperature of the individual affects the acid-base equilibrium of the blood in a way very similar to voluntary over-aeration. (Haggard, 1920; Bazett and Haldane, 1921; Koehler, 1923; Pemberton, Crouter and Cajori, unpublished.) An increase in body temperature brought about by hot baths has been shown to cause a stimulation of the respiratory center with a consequent over-ventilation of the lungs and a reduction of the carbonic acid in the blood. The blood becomes more alkaline (the pH may rise 0.10 to 0.20), the alveolar  $\text{CO}_2$  and  $\text{CO}_2$  content of the blood fall, though the  $\text{CO}_2$  absorption curve may rise. The cH of the urine falls and the excretion of ammonia and acids diminishes. Bicarbonate is excreted in the urine and acetone bodies have been found. Not only do these variations appear when the temperature is raised by artificial means but similar changes may occur in natural fevers. Koehler found in patients with fever the pH of the blood increased 0.10 to 0.15 above the values after recovery.

**EXCESS OF ACIDS.** When an excess of acids floods the body, due either to acid ingestion or to an increased formation of organic acids just the opposite condition is encountered. The stores of base in the form of bicarbonate are drawn upon and the stronger acid displaces the carbonic acid. The carbonic acid is eliminated by way of the lungs, and the non-volatile acid by the kidneys. Some of the base is conserved for the body by the excretion of a portion of the acid as the ammonium salt and a portion as the free acid. The extreme variations in blood and urine in different individuals depend upon the amount of acid introduced and upon the efficiency of all of the regulating mechanisms.

**MUSCLE EXERCISE.** Increased oxygen consumption and carbon dioxide formation are the necessary accompaniments of muscle exercise. To supply the increased quantity of oxygen and to facilitate the removal of the increased quantity of carbon dioxide, the nervous, circulatory and respiratory systems act in coördination (Bainbridge, 1919, Douglas and Haldane, 1922) but do not always succeed in maintaining the normal conditions in the body.

Until recently only isolated observations have been made on the changes in arterial and venous blood before and after exercise. The recent experiments of Lundsgaard and Möller (1923 a, b) Barr, Himwich and Green (1923) and Barr and Himwich (1923 a, b) furnish more details concerning the changes which may occur after short periods of strenuous muscular exercise.

During muscular contraction lactic acid is formed, the larger part of which is synthesized to glycogen and the remainder oxidized to carbon dioxide and water. During strenuous muscle activity lactic acid is formed in large quantities and some diffuses out from the muscle along with the increased quantity of carbon dioxide. The lactic acid, reacting with plasma bicarbonate, forms a salt with the base present and an equivalent amount of free carbon dioxide, which, together with the  $\text{CO}_2$  entering from the tissues, produces a rapid increase in the carbon dioxide tension of the blood. The increased concentration of  $\text{H}_2\text{CO}_3$  and decreased concentration of  $\text{BHCO}_3$  cause an increased cH of the blood. The increased cH of the blood increases the dissociation of oxyhemoglobin (Barcroft, 1914) and favors the passage of more oxygen to the tissues. The loss of oxygen permits the liberation of base from hemoglobin. Thus, the mechanism involving the reciprocal exchange of carbon dioxide and oxygen described as the isohydric change comes into play to an exaggerated degree. But the carbonic and lactic acids from the muscle cannot be completely neutralized by the base released from hemoglobin so the venous blood coming from the muscle has actually a higher cH than normal. During the first minutes of a short period of strenuous muscle exercise the arterial cH and arterial and alveolar  $\text{CO}_2$  tensions are all above normal. The increased cH stimulates the respiratory center, and hyperpnea results with a greater aeration of the blood. The aeration is sufficient to cause a fall in the arterial  $\text{CO}_2$  tension but the continued passage of lactic acid from the muscle into the blood causes the cH of the blood to rise (the pH may fall to 7.05) and the alkali reserve to diminish. In the resting period after exercise the cH returns to normal in a few minutes but the lactic acid is removed more slowly so that the carbon dioxide tension and carbon dioxide capacity may remain low for a half-hour or more.

To counteract the increasing cH of the blood several mechanisms come into play. Lactic acid is taken up by other tissues in the body. In experiments involving strenuous exercise of the legs Barr and Hinwich (1923 a) found that the venous blood coming from the arm contained less lactic acid than the arterial blood going to the arm. The diffusion of lactic acid from the blood releases base so that the carbon dioxide capacity increases and the cH in the venous blood is at times even lower than in the arterial, although both are still above normal. While some lactic acid is being removed from the blood by the inactive tissues some is also being excreted in the urine but the excretion of ammonia and a more acid urine assist in conserving base for the body (Talbert, 1920; Campbell and Webster, 1922 a).

In exercise the temperature of the body is increased (39.0°C. or even higher) which has been shown to be of benefit by making more effective the circulatory and respiratory adjustments during exercise (Bainbridge, 1919). It may possibly be of assistance also in helping to maintain the acid-base balance as it has been shown that a rise of several degrees in body temperature brings about a decreased cH of the blood.

The oxygen content of arterial blood increases during exercise due in part to increased saturation of hemoglobin and in part to an increased oxygen capacity. There is presumably an increased utilization of oxygen in the active muscle but owing to the greatly increased blood flow the oxygen in venous blood is not lowered to values much below normal. The oxygen content of venous blood from inactive muscles is, however, very much reduced due presumably to decreased rate of blood flow.

**LOWERED BAROMETRIC PRESSURE.** The effect of a rapid decrease in barometric pressure as met with in mountain climbing is said to be identical with over-aeration (Hasselbalch and Lindhard, 1916; Haldane, Kellas and Kennaway, 1919-20). Owing to the lack of oxygen the respiratory center becomes more irritable and excessive ventilation results. Carbonic acid is eliminated more rapidly than it is formed, the CO<sub>2</sub> tension is reduced and a diminished cH of the blood presumably results. The cH of the urine is decreased under these conditions and less acid and ammonia are excreted. This picture, as presented by Haldane, is almost the reverse of that originally held by him which postulated the formation of acid metabolic products which caused a lowering of the alveolar CO<sub>2</sub>. It should be noted that the only direct experimental evidence at present of an actual decrease in cH of the blood is the study of the urine.

**BLEEDING.** The uncertainties of such conclusions may be illustrated by observations of Evans (1921 a) and Endres (1922) which have been substantiated by the author (unpublished), on the effects of bleeding. Loss of blood is usually followed immediately by a decreased alkali reserve of the blood. But the animal soon recovers from the immediate effects of the bleeding and, after some hours, the alkali reserve is found to be above normal. The blood at this time has a lower cH than normal and contains more bicarbonate while the urine excreted contains a higher cH and greater titratable acidity and ammonia. In this instance the changes in the urine would appear to indicate a condition in the blood which is just the opposite of that actually occurring. The introduction of this complication in a condition where oxygen want and overventilation may be postulated must render doubtful the interpretation of similar evidence in other conditions.

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## THE INTERNAL SECRETIONS OF THE REPRODUCTIVE ORGANS

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The secretory activity of the generative glands (testes and ovaries) differs from that of other organs of internal secretion in being in a very marked degree cyclical; that is to say, that although the hormones are probably elaborated to some extent at all times especially during the period of reproductive life which extends in the female from puberty until the climacteric, and in the male from puberty until extreme old age, the secretions change both in composition and in amount with certain recurrent seasons which are correlated with the times for breeding. This periodicity is partly inherent in the reproductive organs themselves, but it is also much influenced by external or environmental factors such as nutrition and seasonal and climatic conditions.

THE OVARIAN SECRETIONS AND THE OESTROUS CYCLE. In the female the recurrent changes in the generative organs and the phenomena associated with them characterise what is known as the oestrous cycle, and this is divided into a number of periods which are as follows:

- (1) Anoestrus or period of quiescence.
- (2) Prooestrus or period of "coming on heat."
- (3) Oestrus or period of desire.
- (4) Pregnancy or (alternatively) pseudo-pregnancy.

This is the typical succession in monoestrous animals such as the dog in which there are commonly two sexual seasons in the year and a single oestrus or "heat" period in each sexual season. In polyoestrous animals such as the mare, cow, sheep, pig, rat and mouse, there are several oestrous periods within one sexual season, and these periods are followed by short intervals of comparative quiescence which are known as "dioestrous periods." In such animals pseudo-pregnancy does not usually occur or else is very abbreviated. (The terminology here employed is mainly that originally proposed by Heape.)

The changes which occur during the oestrous cycle relate to the ovaries, the uterus, the vagina and the mammary glands, and there are also general metabolic changes concerning which less is known. The

ovaries contain the controlling factors for all these changes for if these organs are removed the oestrous cycle is brought to an end. If the operation be done prior to puberty the uterus remains infantile and the mammary glands also fail to develop. If the ovaries be removed after puberty when the cycle has started the uterus undergoes atrophy and the mammary glands retrogress unless the operation be done during lactation. On the other hand if the ovaries (or one of them) be transplanted to an abnormal position, notwithstanding the fact that their normal nerve connections are severed, the grafted organ continues to exert its usual influence upon the metabolism, and the oestrous cycle is continued. The assumption is therefore that the ovary exerts its influence upon the other generative organs and upon the metabolism through its internal secretions.

The ovarian elements which seem capable of possessing internal secretory functions are the follicular epithelial cells, the luteal cells of the discharged follicle (these being derived by hypertrophy mainly or entirely from the follicular epithelial cells) and probably also the interstitial cells which are commonly present in the theca interna of the wall of the follicle, in the hilum, and sometimes in the stroma of other parts of the ovary. The interstitial cells constitute the "puberty gland" of Steinach and other authors.

Fraenkel supposed that menstruation in man was brought about through the influence of the corpus luteum. But on the assumption that the menstrual phenomena of the primates are homologous with the prooestrus changes of the lower mammalia, this theory becomes at once untenable, since it has been shown that in the bitch, the sow and various other animals ovulation takes place during oestrus and therefore not until the prooestrus is over; moreover in the bitch the heat periods recur at infrequent intervals (typically once in six months) and a corpus luteum formed from a follicle discharging at one period degenerates before another period is due. It would seem certain therefore that heat in animals and the corresponding processes in man must be brought about by the action of an ovarian secretion arising either in the follicular epithelial cells or in the interstitial cells or possibly in both. In this connection it may be pointed out that according to Lane-Claypon and others the follicular cells and the epithelioid interstitial cells have a common embryonic origin from the primitive germinal epithelium.

Some experiments on the ovaries of bitches (Marshall and Runciman) seemed at first to afford evidence that the presence of mature follicles is not essential for the occurrence of heat. In two animals all the

follicles approaching ripeness, at any rate as far as could be seen, were ruptured artificially by pricking, a few weeks before a heat period was expected, the duration of the oestrous cycle having previously been noted for each individual. Notwithstanding the operation heat occurred in each of the two bitches at about the usual time. The inference at first drawn was that the normal processes of follicular maturation and the phenomenon of heat are both effects of some further factor which must be sought for in the ovaries elsewhere than in the ripe follicle. Interstitial cells are present in the ovaries of the bitch, but they are said to be absent in some mammals (O'Donoghue), and there is no direct evidence that these cells are more active during the heat periods than at other times. Moreover, in two later experiments on bitches, after destroying the mature follicles with a cauterized needle, heat did not supervene at the expected times although the animals had made a complete recovery from the operations, but at a very much later date one of the bitches again came in heat. (Marshall and Wood—unpublished experiments.) Robinson has suggested that in the recorded experiments the cells of the ruptured follicles were not necessarily functionally interfered with notwithstanding the fact that at the time of killing (a short time after heat was over) they had developed into luteal cells. Robinson states further that in the ferret oestrus is experienced only when the follicles are in a certain stage of development which he calls the "pre-inseminal stage." In this animal oestrus may persist for an unusual duration of time in the absence of the male, and during the whole of this period the ovaries contain follicles which remain in the pre-inseminal stage without either discharging or undergoing atrophy. Eventually the follicles with their contained ova degenerate and oestrus passes off. Robinson believes therefore that the mature follicles provide the internal secretion which is responsible for the phenomena of the prooestrus and oestrus. Pugh has recently described what is perhaps a comparable condition in cows and heifers affected with "nymphomania," an abnormal state in which they are continuously "bulling" and always ready to receive the male. Some of the larger follicles become cystic without being septic (as may happen with discharged follicles, which become infected from the Fallopian tubes and uterus) and Pugh suggests that the cystic condition stimulates the growth of the follicular epithelial cells and so favours the production of the internal secretion which is in some way responsible for the continuous oestrus. Lothe also has found that when heat in cows is con-

tinuous or too frequent the condition is very frequently associated with cystic ovaries usually accompanied by endometritis or cervicitis.<sup>1</sup>

The conclusion clearly to be drawn from all these experiments and observations is that the ovarian hormone which produces oestrus or heat is different from that which is responsible for maintaining the normal uterine nutrition.

THE CORRELATION BETWEEN THE CORPUS LUTEUM AND THE UTERUS. The part played by the corpus luteum is much clearer. Broadly speaking this organ is responsible for the changes which take place in the accessory female generative organs and mammary glands during pregnancy and pseudo-pregnancy. We may now briefly consider the evidence for this statement.

In the former times various functions had been assigned to the corpus luteum but the suggestions made were all of them practically devoid of experimental evidence. According to one view it was held that the corpus luteum was the result of excessive vascularization; another theory suggested that the structure was of the nature of a "stop gap" to preserve the cortical circulation of the ovary by preventing an excessive amount of scar tissue; a third theory affirmed that the corpus luteum existed to prevent ovulation during pregnancy. That ovulation does not take place in the presence of a functionally active corpus luteum would seem to be generally true, but it is also true that oestrus does not supervene under such a condition, and it is more in accordance with the facts to regard the suppression of ovulation as of the nature of a consequence rather than a purpose.

Prenant seems to have been the first to suggest that the corpus luteum was a ductless gland which exercised an influence over the general metabolism, but to Fraenkel belongs the credit of assigning to this organ a definite rôle as an internally secreting gland, and basing his theory on definite experimental evidence. According to Fraenkel's theory the corpus luteum possessed the function of elaborating a hormone which in some way assisted in the attachment of the fertilized ovum to the uterine mucous membrane and in the maintenance of its nutrition during the first part of pregnancy. The evidence was derived mainly from the results of experiments in which the ovaries were removed or the

<sup>1</sup> The writer has examined a section, kindly sent him by Mr. Pugh, of an ovary of a nymphomaniac cow and this shows an unusually large number of capillaries running through the stroma. Epithelioid interstitial cells are also present in some number, besides follicular epithelial cells in normal follicles of various stages of growth.

corpora lutea destroyed, and in each case the pregnancy was brought to an end. Control experiments proved that the effects were not merely post-operative. The theory was afterwards extended to explain the cause of menstruation and "heat" and the various phenomena which other investigations had attributed to the ovarian secretion, but as already indicated, this view as to the rôle of the corpus luteum cannot be made to apply to the lower mammals with infrequently recurring periods of oestrus. Moreover, although the original part of Fraenkel's theory is now generally accepted, it is not justifiable to regard the corpus luteum as the responsible factor in the attachment and nutrition of the ovum or early embryo in any other sense than that implied in the statement that this organ, through the secretion it produces, acts as a stimulus to the growth of the uterine mucosa and the maintenance of the increased uterine nutrition which are necessary for the occurrence of gestation. Fraenkel's experiments on ovariectomy and the destruction of corpora lutea have been confirmed by numerous other investigators, but it was not until Ancel and Bouin had shown that the corpus luteum exerts a comparable influence on the rabbit's uterus in pseudo-pregnancy that the general conclusions regarding the function of that organ were placed on a completely firm foundation.

As Heape showed long ago, the rabbit normally ovulates only after coition. Consequently the presence of corpora lutea in the ovaries of that animal is usually correlated with the occurrence of pregnancy, and the so-called corpora lutea spuria in the rabbit do not exist. If, however, pregnancy is prevented through coition being made sterile, as by submitting the male to vasectomy, then ovulation takes place and corpora lutea are formed unaccompanied by gestation. Ancel and Bouin were the first to show that in such circumstances the uterus undergoes growth, glandular increase and vascularization followed later by retrogressive changes, and that these changes are clearly correlated with the development and subsequent decline of the corpora lutea in the ovaries. Moreover the uterine changes present a general similarity to those occurring in pregnancy, but decidual tissue is not normally formed.

Hill and O'Donoghue have described what are evidently comparable changes, but occurring normally after oestrus, in the non-pregnant marsupial cat (*Dasyurus viverrinus*). These authors were the first to use the name "pseudo-pregnancy" in this connection, though the term had been previously applied to describe an abnormal condition resembling pregnancy in man, by Matthews Duncan. In the marsupial

cat ovulation takes place spontaneously and there is only one sort of corpus luteum formed irrespectively of whether pregnancy or pseudo-pregnancy supervenes. During pseudo-pregnancy the uteri enlarge and become considerably vascular, and these changes are succeeded by degeneration and desquamation of epithelium with an extravasation of blood in just the same kind of way as happens in the experimentally produced condition of pseudo-pregnancy in the rabbit. Eventually regeneration sets in and the mucous membrane undergoes recuperation.

The dog also has a normal pseudo-pregnant period if ovulation (which in this animal is likewise spontaneous) is not succeeded by true gestation. The development of the uterine glands is very similar to that which takes place during pregnancy, but in the latter condition the secretion formed is a source of nutriment to the fetus. The entire sequence of changes is correlated with the contemporaneous series of ovarian changes, the corpus luteum undergoing a degree of development comparable to what occurs in gestation and persisting for a period nearly or quite as long (Marshall and Hahnan).

In the other mammals for which the changes during the oestrous cycle have been described there is either no pseudo-pregnant period or else it is very much abbreviated in correlation with the shortened period of persistence of the corpus luteum which after the dioestrus or short period of sexual rest, makes way, so to speak, for a new heat period and a new ovulation. In the sow Corner has described post-oestrous activity on the part of the uterine glands for eight or nine days, after which the epithelial cells slowly revert to a condition characteristic of heat. The changes therefore are suggestive of a shortened pseudo-pregnancy occurring under the influence of the corpus luteum which undergoes retrogressive changes in the later part of the dioestrus. It is possible also that the growth stages of the menstrual cycle of man represent partly a pseudo-pregnant growth and that the destruction stages similarly are not simply prooestrous but correspond to some extent to pseudo-pregnant regression, owing to the whole cycle of changes being compressed into one month. Lipes states that in man the constructive uterine stage commences as soon as post-oestrous repair is completed, and there is often great glandular development. Moreover, according to Hitschmann and Adler, the premenstrual uterus undergoes changes which are similar in character to those observed in the pregnant uterus, so that it is possible to regard these as taking place under the influence of the corpus luteum.

However this may be, it seems certain that the post-oestrous uterine changes in the non-pregnant marsupial cat, bitch and rabbit (in the latter animal only usually occurring under experimental conditions) are physiologically homologous, and that they take place under the influence of the corpus luteum, since apart from the observed relation between the growth and regression of the corpus luteum on the one hand, and the uterine mucosa on the other, the phenomena of pseudo-pregnancy in the rabbit only supervene after a sterile coition which induces ovulation and admits of the subsequent formation of the corpus luteum.

The existence of a uterine cycle correlated with the ovarian cycle has also been shown in the case of the guinea pig by Leo Loeb.

Decidual cells are not normally formed in the uterine mucosa excepting in true pregnancy, but as was first shown by Loeb in the guinea pig nodules composed of decidual tissue can be induced to develop under the influence of direct stimuli to the mucosa such as the introduction of a foreign body into the uterine cavity or the making of a number of incisions in the mucosa so as to break the continuity of the tissue. The nodules which Loeb describes under the term "deciduomata" arise through the proliferation of the inter-glandular connective tissue. They can be induced to form most readily from the third or fourth to the eighth or ninth days after heat and therefore at a time when freshly formed and active corpora lutea are present in the ovaries. The formation of decidual tissue was not caused by ova in the uterus, since it took place when that organ was ligatured off so as to prevent the passage of the ova. If however the ovaries with their contained corpora lutea are extirpated deciduomata are not produced. On the other hand, if pieces of uterine mucosa are transplanted into subcutaneous tissue decidual nodules are formed in the grafted tissue. Loeb concludes therefore that for a certain interval after ovulation the corpora lutea elaborate a predisposing substance in the presence of which indifferent stimuli may produce the formation of deciduomata.

Hammond has shown further that placental tissue may be formed in the uterine mucosa of the rabbit by similar methods but only during an experimentally induced pseudo-pregnancy. Such a formation of decidual tissue is clearly comparable to that produced during true pregnancy when corpora lutea are normally present in the ovaries.

Long and Evans state that in the rat owing to the short dioestrus and the corresponding abbreviation in the duration of the "corpus luteum spurium" or "corpus luteum of ovulation" deciduomata cannot

be induced in the uterine mucosa. If however the female rat undergoes a sterile coition with a vasectomized male the corpus luteum persists for a longer period and the subsequent oestrus is postponed. This is believed to be due to the formation of the vaginal plug which extends into the cervical canal of the uterus and has a direct stimulating effect on the mucosa, producing a condition of pseudo-pregnancy, and the corpus luteum itself persists for a longer period. The same result can be brought about in the absence of the male by mechanical stimulation of the tissues at the anterior end of the cervix by a tube or glass rod. Further if during pseudo-pregnancy the uterine mucosa were subjected to irritation induced by injury or by the introduction of fine threads into the uterine cavity deciduomata were formed in just the same kind of way as with the guinea pig or the rabbit. It is believed therefore that the internal secretion of the persistent corpus luteum sensitizes the uterine mucous membrane, thereby rendering it capable of reacting to mechanical stimulation in the rat just as it has been shown to do in the other animals experimented upon. In the pregnant animal in which the corpus luteum also persists the direct stimulus is produced by the fertilized ovum.

THE CORRELATION BETWEEN THE CORPUS LUTEUM AND THE MAMMARY GLANDS. That the growth of the mammary glands is dependent upon a stimulus derived from the corpus luteum was first shown by Ancel and Bouin in the rabbit. In the virgin rabbit the mammary tissue is limited to a few ducts in the immediate neighborhood of the nipple. After ovulation however growth proceeds rapidly both in pregnancy and in pseudo-pregnancy and at a certain stage of development of the tissue the hypertrophy is sufficient to allow of secretory activity. As already stated ovulation in the rabbit depends upon coitus, so that the pseudo-pregnant hypertrophy of the mammary glands only takes place ordinarily under experimental conditions such as when a vasectomized buck is used. In the marsupial cat, as shown by O'Donoghue, pseudo-pregnant mammary development followed by milk secretion takes place also, but in this animal ovulation is spontaneous. The bitch is similar. Since however the post-oestrous development of the mammary glands (like the synchronous hypertrophic changes in the uterine mucosa) only takes place in the rabbit in the presence of corpora lutea, and since the parallel series of changes in *Dasyurus* and the dog are also always associated with the development of luteal tissue, there can be no doubt that the corpus luteum is an essential factor for the growth of the milk glands in all these animals. Moreover, the commencement of milk

secretion is marked by retrogressive changes in the corpus luteum. Leo Loeb states that in the guinea pig the corpora lutea are responsible for the secondary growth of the mammary tissue which occurs in the later period of gestation.

In the rat the corpus luteum formed as a result of ovulation occurring immediately after parturition is described by Long and Evans as the "corpus luteum of lactation," and it would appear that in this animal at any rate luteal tissue may undergo development at the same time as actual milk secretion, and it is well known that the same process occurs to a limited extent in such polyoestrous animals as the cow. It would appear possible therefore that the anabolic changes involved in the building up of mammary tissue and the katabolic changes concerned in actual milk secretion may go on simultaneously, and that the corpus luteum may exert an influence on both phenomena which are to be regarded as parts of one process. The fact first discovered by Ott and Scott that luteal extract injected into the circulating blood causes an almost immediately outpouring of milk is evidence supporting this view.

THE CORPUS LUTEUM IN POLYOESTROUS ANIMALS AND THE PERSISTENT CORPUS LUTEUM. It is well known that in polyoestrous animals such as the mare, the ewe, the cow and the sow the corpus luteum persists for only a short time if pregnancy does not supervene after oestrus. This, to speak teleologically, is to admit of heat and ovulation recurring after a short interval, since these processes cannot ordinarily take place in the presence of a fully developed functional corpus luteum in either ovary. It would appear that the corpus luteum when functionally active dominates the ovarian metabolism and inhibits the formation of the internal secretion which is an essential factor in producing prooestrus and oestrus besides hindering the maturation and rupture of the Graafian follicles. In monoestrous animals like the dog, on the other hand, the corpus luteum persists during a pseudo-pregnant period which may be as long in duration as the period of true gestation, since in such animals a new heat period is not due in any case until many months have elapsed after the previous ovulation period. Reference may be made in this connection to the observations of Heape, Noël Paton, Blair Bell and others of non-pregnant bitches, in many cases virgins, secreting milk at about the time when they would have given birth to pups had they become pregnant.

Even in polyoestrous animals, however, provided that ovulation takes place spontaneously in oestrus, the mammary glands may be sufficiently built up during a succession of dioestrous cycles as to admit

of milk formation at a later stage, for Woodman and Hammond, and more recently Asdell (unpublished work), have found that a fluid can be drawn off in some abundance through the teats of virgin heifers, and that the fluid so obtained contains lactose and all the essential constituents of milk. The degree of development and functional capacity of the mammary glands of such animals is in striking contrast to those of the virgin rabbit in which corpora lutea are not formed and in which consequently development of mammary tissue does not take place.

The cat resembles the rabbit in only ovulating after coitus, as shown by Longley, but Doncaster has described a female which after copulating with a sterile tortoiseshell male underwent mammary hypertrophy for about a month and to an extent sufficient to result in secretion of milk which lasted for two weeks. This was clearly a case of pseudo-pregnancy comparable to the condition occurring in the doe rabbit after copulating with a vasectomized buck.

It has been mentioned that in the presence of the corpus luteum ovulation does not take place. In confirmation of this Leo Loeb found in the guinea pig that ovulation rarely occurs within sixteen to eighteen days after a preceding ovulation, but that if the corpora lutea are removed from the ovaries the ovulation interval may be reduced to from twelve to six days. Again, according to Pearl and Surface, the desiccated fat-free extract of cow's corpus luteum when injected into a laying fowl, immediately inhibits ovulation, but after ceasing the injections ovulation and laying proceed as before. Pearl and Surface state that the substance which produces this result is rendered inactive by boiling.

Under certain abnormal conditions the corpus luteum of the non-pregnant cow or heifer may persist for a prolonged period. Such a condition is usually associated with endometritis or some pathological affection of the uterus or Fallopian tubes, and sterility with non-occurrence of oestrus results. That the non-occurrence of oestrus is due to the persistent corpus luteum is proved by the observations and experiments of Zschokke, Williams, Hess, Oppermann, Pugh, Lothe and others who have shown that if the corpus luteum be removed or destroyed oestrus will generally recur within from 3 to 8 days, and the animal may be got to breed. The results of these and other experiments carried out upon cows in veterinary practice are thus in strict conformity with what is known concerning the influence of the corpus luteum in the other animals in which the ovarian and correlative processes have been investigated and described.

**THE OVARIES AND PARTURITION.** The study of pseudo-pregnancy in the bitch, the experimental rabbit and the marsupial cat throws some light on the factors responsible for parturition. In each of these species there is present at the end of pseudo-pregnancy a persistent corpus luteum in a condition of involution not dissimilar to that of the corpus luteum verum at the end of true pregnancy. Further, pseudo-pregnancy can only occur when a corpus luteum is present. Moreover, all these animals display habits and instincts at the end of pseudo-pregnancy which are identical with or similar to those associated with parturition. Thus the bitch may prepare a bed as if for a litter of pups, the doe rabbit plucks her breast of fur and uses it to line a nest (Hammond), and the female marsupial cat cleans out her pouch as though for the reception of young (Hill and O'Donoghue). It has been shown that the occurrence and duration of pseudo-pregnancy are dependent on the corpus luteum, and consequently it is exceedingly probable that the processes associated with parturition are similarly correlated with changes in the amount or character of the ovarian secretions. Ancel and Bouin suggested that whereas in the first part of pregnancy the tolerance of the uterus for the fetus was due to the corpus, during the second part it was brought about through the "myometrial gland" of the uterus, but Hammond has shown that it is far more likely that the corpus luteum is the responsible organ throughout the whole of gestation, since this organ persists and is apparently functionally active until quite a late stage in pregnancy, whereas in pseudo-pregnancy the corpus luteum and mammary glands are never so completely developed and the former organ does not retain its state of maximum growth so long. Furthermore Sharpey Schafer has shown that the ovaries may produce at least two kinds of hormones which act differently on plain muscle, one increasing contractility and the other acting as an inhibitor. The respective amounts of these secretions probably vary at different stages of the cycle and may show a relation to different phases in the history of the follicle and corpus luteum, but further evidence is needed before such a theory can become anything more than a suggestion. Very recently Dixon has carried out some experiments which are calculated to throw further light upon this question. It is well known that pituitary extract promotes uterine contraction, and Dixon has shown that ovarian extract (without corpus luteum) when injected into the circulation causes pituitary secretion, but that corpus luteum extract has no such effect. It is possible therefore that during pregnancy when the corpus luteum dominates the ovarian metabolism, the activity of the normal

ovarian secretion is reduced, but that at the end of pregnancy when the corpus luteum has entered into a state of involution, the ovarian secretion reasserts its influence, and by activating the pituitary promotes those uterine contractions which are the cause of parturition.

THE CORRELATION BETWEEN THE OVARIES AND THE SEXUAL CHARACTERS. As already mentioned, there is an undoubted functional correlation between the ovaries and the normal nutritional condition of the uterus since ovariectomy is followed by uterine degeneration which may be arrested by the successful transplantation of an ovarian graft in an abnormal position. There is some evidence that the ovarian interstitial cells are responsible for elaborating the internal secretion which is responsible for maintaining the normal nutrition of the uterus, for McIlroy found that this is preserved by ovarian grafts in which the follicle cells have degenerated and, of the possible secretory elements, only the interstitial cells remain.

The study of the distribution and comparative physiology of the ovarian interstitial tissue is still however very imperfect and in some animals interstitial cells have not been discovered, at any rate in the ovaries of the adult. Thus, according to Aime, ovarian interstitial cells have not been seen in the sheep, pig, dog or man or in the adult horse, whereas they are stated to be present in the adults of bats, insectivora and rodents, in both the fetal and the adult cat, and in the fetal horse. Robinson states that he has seen them in the dog. O'Donoghue has observed them in marsupials; and van der Stricht and Athias in bats. Cese-Bianchi and Rasmussen have described them as undergoing cyclical changes in hibernating animals such as the woodchuck, becoming most active during the times of sexual activity and becoming much reduced during the winter sleep. Regaud and Dubreuil and Wallart state that the interstitial cells increase during pregnancy. Evans, referring to the rat, says that there is no hypertrophy of the interstitial tissue at puberty. It is not unlikely that there is considerable variation among the different species of mammals in regard to the development and functional importance of these cells, and it must be remembered that according to Lane-Clayton and McIlroy the follicular epithelial cells and interstitial cells have an identical origin and are therefore probably potentially equivalent.

According to Steinach and his followers, the interstitial cells of the ovary represent the "puberty gland" of the female organism, that is to say, that gland which is responsible for all the essentially female characteristics including not only the accessory generative organs such as the

uterus and mammary glands but also the secondary characters of sex and the psychological female characters. The organ is called the "puberty gland" because those characters which depend upon its activity undergo marked development at puberty.

This view is based upon a large number of experiments and observations upon various species of mammals. Male animals are described as becoming "feminized" by the introduction of transplanted ovaries after the previous removal of the testes. Guinea pigs operated upon and treated in this way are stated to have undergone remarkable development of the mammary glands and teats, and to have produced milk and even to have suckled the young of other individuals. The hair of these feminized animals is said to have resembled that of normal females, being finer and softer than in the male. Moreover, the guinea pigs with successfully transplanted ovaries showed typical female reactions, such as the "tail-erect reflex" normally concerned in coitus, and the "kick-guarding reflex" which is employed by the female to ward off the male prior to the onset of oestrus. Athias, Moore and Sand have also described development of mammary tissue and milk secretion in animals (rats and guinea pigs) after the introduction of ovarian grafts.

Steinach and Holzknecht have studied the effects of Röntgen ray treatment on the ovaries. They state that these showed a degeneration of follicles but a survival or even accentuation of interstitial cell growth. Moreover, the increase in the interstitial tissue following a correct x-ray dosage resulted in mammary growth and eventually in milk secretion. In women one of the effects was a postponement of the climacteric. All these results are attributed to the activity of the female puberty gland which is supposed to preside over the metabolism of the accessory sexual organs and the secondary female characters. It has been shown however (as described above) that mammary development is normally due to the activity of the corpus luteum, and if the interstitial cells have any influence over the milk glands in feminized animals it is perhaps comparable to the slight hypertrophy which takes place during the prooestrus before ovulation or possibly to the primary development of the gland at the beginning of pregnancy which, in the guinea pig, Leo Loeb distinguishes from the more pronounced secondary hypertrophy occurring later under the influence of the corpus luteum. Otherwise one must assume that under certain conditions the interstitial cells act vicariously for the corpus luteum.

The experiments by Steinach and others on the masculinization of females are referred to below in dealing with the male "puberty gland."

That the ovaries are functionally correlated with the development of the secondary female characters is more apparent in birds than in mammals, since in birds the neutral type is outwardly much nearer to the male than to the female, and there is little or no evidence that ovariectomy in any animals results in the development of distinctively male characters. Tandler and Keller state that removal of the ovaries in heifers produces a type similar to that of the castrated male, the head resembling the head of the steer. In Herdwick sheep which are horned in the male and hornless in the female, ovariectomy is not followed by the growth of horns (Marshall). In other species of mammals the operation is negative in its effect upon the bodily conformation and appearance.

In birds, on the other hand, ovariectomy is followed by very pronounced results. Goodale and Pézard have shown that in fowls it produces birds similar or identical with castrated males. Thus with Brown Leghorns the spayed hen assumes the plumage of the Leghorn cock, with red back, black breast and long pointed hackle and saddle feathers, and spurs develop on the legs. The sickle and other characteristically male feathers, however, did not appear, and the comb and erectile structures did not hypertrophy as they do in the male. Goodale also removed the ovary from the Rouen duck and found that the bird to a very great extent assumed the plumage of the drake. Duerden and Fitzsimons record that ovariectomy in the ostrich is followed by retention of the ordinary body color, but that the normally grey feathers assume the black coloration of the cock, a fact which has been taken advantage of for commercial purposes by South African ostrich farmers who make a practice of spaying certain of the hens. Zawadowsky has described the effect of ovarian ablation in pheasants, and these are essentially similar to the de-sexed females presenting a close superficial resemblance to cock birds of the same species.

Such observations as these have led Lipschütz and Pézard to elaborate the idea of a neutral or indifferent type upon which the internal secretions of the generative glands operate, and it is suggested that even in the embryo there are sexual endocrine organs which are responsible for initiating the growth of the distinctively male or female characteristics. This idea receives support from Minoura's experiments on chicks. This investigator removed portions of the egg shells during the second week of incubation and transplanted onto the chorio-allantoic membrane portions of gonads from other individuals, and by this method succeeded in producing different grades of intersexuality. The grafts

were obtained both from other chicks and from older birds but the effects were the same, and in female-type embryos the right ovary, which usually atrophies with birds, might be got to persist as a result of a successful ovarian graft. Further experiments dealing with this subject are described below in considering the internal secretory activities of the testis.

It is not known what precise ovarian elements are responsible for the production of the hormone which is an excitant for the development of the female organs and characters or the possible inhibition of the distinctively male ones, and in birds there is even less evidence upon this question than there is in mammals (cf. Hartman and Hamilton).

THE INTERNAL TESTICULAR SECRETION AND THE MALE GENERATIVE CYCLE. In most male mammals there is a rutting season which occurs at the same time as the sexual season in the female, and spermatogenesis is confined to this time. The periodic activity of the testis is usually correlated with a great increase in the size of that organ. Thus in the mole according to Régaud and Lécaillon the testicles increase in bulk sixty-four times.

As is well known, the growth of the accessory male organs and the secondary male characters is dependent upon the presence of the testes, and this is true not only of the pubertal growth but of the periodic or seasonal development where such occurs. Thus the vesiculæ seminales, the prostate and other accessory sexual organs of the adult male hedgehog which undergo an enormous development periodically with the approach of rut, fail to do so after the removal of the testes (Marshall), and it is well known that the antlers of the stag do not undergo their annual growth if the animal be previously castrated, notwithstanding that the stag may have reached maturity and grown antlers in the previous year before the operation was performed. Again in Herdwick rams the presence of the testes is not only necessary for the initiation of horn growth but also for its continuance, since the horns cease to grow forthwith after castration and at any stage of development (Marshall). That the testis exerts its influence through the medium of the circulating blood and therefore by means of an internal secretion is proved by the effect of testicular grafts in abnormal positions in just the same kind of way as with the female. This was first definitely shown by Berthold in 1849.

The glandular substance of the testis consists of the spermatogenetic tissue contained within the seminiferous tubules (together with the cells of Sertoli) which are generally believed to have a nutritive or supporting

function connected with the production of the reproductive cells, and the interstitial tissue consisting of the cells of Leydig which lie outside of and between the tubules and are collectively included by Steinach under the term "puberty gland." They are believed by this physiologist to correspond in the male to the interstitial or puberty gland of the ovary. There has been a considerable amount of controversy as to the seat of production of the internal testicular secretion, but the bulk of the evidence seems to show that it is produced exclusively by the interstitial cells, at least among mammals; indeed the evidence is far clearer in the case of the male than it is for the female.

Bouin and Ancel appear to have been the first to show that when in the horse and other animals the vasa deferentia are ligatured the spermatogenetic tissue of the testis gradually ceases to be functional and eventually undergoes degeneration. This result is possibly due to the semen failing to escape and so exercising an inhibitory influence by back pressure upon the tubules where sperm production consequently ceases. The interstitial cells however, since they are outside the tubules, do not degenerate. Copeman working upon rats obtained similar results. Moreover Shattock and Seligmann found that in Herdwick rams, notwithstanding the degeneration of the spermatogenetic tissue brought about by occluding the vasa deferentia the horns which, in this breed characterize the male, grew normally. Subsequently Tandler and Gross described the effects of subjecting the testes of the roebuck to the Röntgen rays. They found that the spermatozoa and spermatogenetic tissues are destroyed but the interstitial tissue remains unaffected, and in correlation with its presence the horns of the roebuck undergo development as in the normal male. Evidence pointing in the same direction is furnished by those cases of cryptorchism where the undescended testicles come to consist of interstitial tissue only and yet sexual desire is manifested and the secondary male characters are developed. Lipschütz has shown that in the guinea pig a portion of testicular tissue one-sixteenth of the normal size and consisting mainly of interstitial tissue may suffice to admit of the development of the secondary sexual characters. The evidence that the interstitial cells are functionally responsible for the production of the testicular hormone, and that the germinative cells play no part in the process, at least in mammals, seems to be very strong.

That there is an interstitial gland before birth was shown in the horse by Bouin and Ancel who state that it diminishes in the later stages of intra-uterine life, the formation of new interstitial tissue beginning

some time after birth, but not being completed until spermatogenesis occurs. Aron has recently recorded similar observations in the sheep and pig. Lipschütz and others have suggested that this fetal interstitial gland is responsible for the early sexual differences, a conclusion which receives some support from Lillie's work on the free-martin and Minoura's transplantation experiments with chicks. Furthermore Lillie states that Bascom, working in Lillie's laboratory, has found interstitial cells of the same character as those of the adult and filled with secretory granules in the fetal testis of the bull-calf at a stage of development represented by an embryo length of about thirty millimeters.

It has already been mentioned that in the hedgehog the accessory male organs undergo a very pronounced periodic growth after hibernation and with the approach of the breeding season. This is associated with a great testicular development which affects not only the spermatogenetic tissue but also the interstitial cells; indeed the latter proliferate even more than the spermatogenetic cells and the tubules come to be widely separated. Similar facts have been recorded for the mole (Lécaillon) and the woodchuck (Hansemann, Rasmussen).

The remarkable periodic increase in the size of the testis of birds has long been known but the question as to the seat of production of the internal testicular secretion in birds is still an open one. Des Cilleul stated that the appearance of the interstitial cells in the cock coincided with that of the secondary sexual characters, and that the development proceeded synchronously, but other observers have taken a different view. The divergence appears to relate mainly to the question as to whether the so-called interstitial cells in birds are really internally secretory in the sense in which they appear to be in mammals. Thus Boring states that interstitial tissue is abundant in the testes of newly hatched chicks but that there is no evidence that it produces an internal secretion. Reeves found interstitial tissue in young cocks five and one-half, nine and eighteen months old. Pézard maintains that the internal testicular secretion is produced by the germinative cells or the cells of Sertoli. On the other hand Watson found in the greenfinch that there were definite epitheloid interstitial cells but that they showed their most pronounced development in the non-sexual season, and that as spermatogenesis approached they decreased in number. More recently Massaglia, as a result of experiments on the effects of ligation of the vasa deferentia, found that there was a degeneration of the spermatogenetic tissue but no such effect upon the interstitial cells,

and that in correlation with this the secondary male characters were normal. These experiments, therefore, were similar both in method and result to those with the mammals described above. Nevertheless, Stieve working on the jackdaw states that there is no increase in the interstitial cell growth in correlation with the development of the male characters, and is disposed to deny any endocrine function to the interstitial tissue of the testis.

Among the lower vertebrates Aron has adduced evidence of a correlation between the periodic changes of a peculiar testicular gland and the recurrence of oestrus in the newt (*Molge cristata*). The gland is situated near the hilum of the testis; it develops at the commencement of the sexual season when its cells proliferate, their protoplasm becoming filled with large fatty granules; it persists until the time when the nuptial characteristics begin to disappear when the gland also undergoes retrogression. It had already been demonstrated that after castration the male nuptial characters do not make their appearance but Aron found that the same result could be brought about by the destruction of the special gland above referred to at the beginning of heat, notwithstanding the fact that the seminiferous tissue of the testis was not interfered with. If the gland was only incompletely destroyed then the nuptial changes proceeded normally. Aron's conclusions have been criticized by Champy, but there nevertheless appears to be a strong presumptive case that the gland in question is physiologically homologous with the interstitial gland of mammals.

Among fishes it has been shown by Courier that in *Gasterosteus aculeatus* the testicular interstitial cells assume a glandular appearance at about the time of the nuptial transformation and after spermatogenesis has taken place. In the absence of this change on the part of the interstitial gland the typical development of nuptial characteristics does not occur. Moreover Courier concludes that the interstitial gland cannot be of the nature of a trophic organ for the spermatogenetic cells, since these undergo development before the interstitial transformation. The general conclusion reached is that the interstitial gland in fishes is homologous with that described by Aron for amphibians and with the mammalian interstitial gland of Anceel and Bouin.

THE TESTICULAR HORMONE CONSIDERED IN RELATION TO SEX DIFFERENCES. It will have been seen that there is strong evidence that the interstitial tissue of the testis produces an internal secretion which is responsible for the developmental changes which occur in the male at puberty as well as for the periodic changes associated with the male

generative cycle, including both those which relate to the accessory generative organs and those shown by the secondary sexual characters. Steinach and his co-workers have gone further and, as a result of a large number of experiments, mostly on testicular transplantations into females, ascribe to the interstitial gland a definite sex-determining influence.

In experiments upon guinea pigs and rats it was found that when the testes were transplanted into young females from which the ovaries had been removed, the clitoris developed into a penile structure and there were manifestations of sexual desire as displayed by males; moreover the animals grew to the size of males. Such individuals are regarded as being sexually inverted or masculinized in just the same way as in the converse experiments males with ovarian grafts apparently became feminized. In such animals only the interstitial gland survived. Lipschütz has studied the anatomy of the hypertrophied clitoris in the inverted guinea pig and found that it contained corpora cavernosa as well as the quill-shaped horny spikes which are characteristic of the penis in that animal. Steinach's experiments have been confirmed and extended by Sand and by Moore who however state that they were able also to produce experimental hermaphrodites, testes being transplanted successfully into entire females and ovaries into entire males when the gonads of each kind exerted an influence on the appropriate accessory sex organs without causing a degeneration of the organs correlated with the opposite sex.

Steinach's theory that the internal secretions of the gonads and probably of the interstitial gland are the principal factor in sex determination, receives strong support from the observations made by Tandler and Keller, and by Lillie, on the "free-martin." These investigators showed independently but about the same time that the bovine free-martin is a partial hermaphrodite or rather an inter-sexual individual which started as a female but became modified during embryonic development. They observed that in such an animal the blood vessels of the chorion anastomosed with those of its fellow, that is, with the vessels of the co-twin which after birth becomes a normal fertile bull. The generative organs of the male were shown to be in a more advanced stage of development than those of the female at the same age, and the conclusion was drawn that the hormone produced by the fetal testis exerted an influence over the organs of the originally female twin at an early stage before the ovary as an internally secreting organ was functionally active. The testicular hormone coming from

the normal male twin was of course carried in the common circulating fluid through the united vessels of the two chorionic membranes. Furthermore, as already mentioned, Bascom has found a well-developed interstitial gland in the testis of the bull embryo at the stage in question. Lastly, Lillie found that the two embryos (free-martin and normal male) arise from different ova as proved by the presence of two corpora lutea in the ovaries, and not from a single ovum as had been previously suggested by Berry Hart and others.

This theory of the free-martin has received confirmation from Minoura's experiments upon transplanting gonads of chicks. As related above, Minoura removed a portion of the shell of the incubating egg and grafted on to the chorio-allantoic membrane a piece of gonad from an individual of the opposite sex, and in this way obtained artificial free-martins or inter-sexual individuals among fowls. All degrees of inter-sexuality were produced. Inter-sexes among pigeons obtained by injecting extracts of gonad as well as by other methods have been described by Riddle. The whole of this work together with many other instances of inter-sexuality among various groups and species of animals is well reviewed by Goldschmidt in his recent work on sex-determination.

The effects of gonadectomy in various vertebrates are also in general accord with Steinach's views on sex-determination. This is seen very strikingly in birds in which it has been shown by Goodale, Pézard, Zawadowsky and others that the result of castration in either sex is to produce a neutral type which in this class is much closer to the normal male than to the normal female, the exact converse being the case in mammals. Thus, removal of the testis in the cock has little effect upon the general plumage or the spurs but the comb and other erectile structures do not develop. In pheasants the results are similar. Ovariectomy on the other hand, as already mentioned, is followed by an assumption of the plumage usually associated with the male and by a growth of the spurs, but the erectile organs do not hypertrophy. Similarly in the ostrich Duerden has shown that gonadectomy in either sex results in similar individuals. Among mammals also Tandler and Keller state that removal of the gonads from cattle produces a convergence of type, this being seen especially in the shape of the head. All these observations are an indication that the gonads play a very important rôle, if not indeed the main one, in determining which sex is to develop, thus confirming the view that under certain conditions the chromosome constitution normally associated with a particular sex can be overridden and the opposite sex produced.

THE "PUBERTY GLAND" AND REJUVENESCENCE. The idea of a connection between testicular influence and rejuvenescence, originally put forward by Brown Séquard as part of his general theory of the metabolic effects of the internal secretions of the gonads, has been brought into prominence again recently by Steinach and some other writers with special reference to the interstitial or puberty gland. Thus it has been claimed by Voronoff that successful transplantation of testicular interstitial tissue into the aged has been followed by rejuvenation and generally beneficial results, and the grafting of interstitial gland obtained from apes has been recommended. Voronoff records further a large series of experiments upon goats, sheep and other animals. Successful testicular transplantation in man had been previously recorded by Steinach, Lichtenstern, and others.

According to Steinach, rejuvenation can also be brought about by vasectomy or ligaturing of the vas deferens, operations which result sooner or later in the atrophy of the spermatogenetic tissue without interfering with the interstitial tissue. It is not clearly understood why the spermatogenetic tissue should be destroyed as a consequence of the operation, and the results obtained by various observers are by no means uniform. Thus in testicular grafts in fowls spermatogenesis is known to continue for some time at any rate after the transplanted organ has become attached and without there being any exit for the seminiferous fluid or spermatozoa. There can be no doubt however that vasectomy does frequently, if not usually, result in cessation in the production of the spermatozoa and sooner or later in the degeneration of the seminiferous tubules as shown originally by Ancel and Bouin. (Cf. Myers, etc.) Steinach goes further and states that as a consequence of the changed conditions in the testis the interstitial tissue undergoes hypertrophy and the resulting rejuvenation of the organism is attributed to this hypertrophy on the part of the puberty gland. The operation has been done upon aged men, both bilaterally and unilaterally, and is stated to have been followed by favorable results. The advantage of the unilateral operation is that one testis remains fully functional.

Steinach found that normal unoperated rats seldom lived for more than twenty-six months but that individuals in which the vas was cut or ligatured might live for thirty-six months, and this increase in the longevity is attributed to the hypertrophy of the puberty gland. It must be pointed out, however, that Slonaker in an investigation upon the natural life of the rat found that the normal duration was about

forty months and Donaldson says that the average duration is only slightly less.

Romeis states that he could find no histological evidence that ligation of the vas brought about any augmentation in the interstitial cells of the testis, and that the increase in size on the part of the vesiculae seminales and prostate which occurred (as in Steinach's experiments) was due to post-operative stasis and was not a true functional hypertrophy.

On the other hand, in confirmation of Steinach, Sand has recorded an experiment upon a dog which showed all the signs of senility but after the operation of bilateral vasectomy was restored to a condition of physical robustness and generally rejuvenated. Harms also has described experiments upon other animals with similar successful results.

A possible fallacy underlying all these experiments is that the result of any nutritional or other environmental influence is not easy to determine and may be neglected while effective control experiments are a matter of difficulty. Moreover, in the case of operated men it is always possible that the results are in part at least due to suggestion.

Mottram and Cramer have recently carried out a series of experiments upon rats in which the testes were irradiated over long periods by successive small doses. They found that the animals put on weight more rapidly than the controls and became very obese whereas castrated rats did not put on fat. The seminiferous tubules underwent intense atrophy and apparently as a result of reduced pressure which admitted of a freer blood supply the interstitial cells became hypertrophied. As a consequence changes (atrophic in their essential nature) were produced in the intermediate and posterior portions of the pituitary. The authors put forward the view that in dystrophia adiposogenitalis where the primary lesion is in the pituitary, the sequelae are first, atrophy of the seminiferous tubules, secondly, hypertrophy of the interstitial tissue, and thirdly, adiposity. It is suggested further that deposition of fat occurring in a senile organism as a consequence of interstitial hypertrophy may be the real basis for the claims of rejuvenation made by Steinach and others in the experiments and observations recorded above. Cramer and Mottram however found that vasectomy in rats was not followed by degeneration of the seminiferous tubules or hypertrophy of the interstitial tissue.

The question, therefore, as to the supposed rejuvenating influence of vasectomy and the hypertrophy of the interstitial gland is still an open one, but there can be no doubt that in mammals the secretion

elaborated by this organ is responsible for the development of the pubertal characters, while there is accumulating evidence that the predecessor to this gland in fetal life is a very important factor in sexual differentiation before birth.

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## CHEMOTHERAPY

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I. INTRODUCTION. By introducing the name "Chemotherapy" Ehrlich intended to mark off, from the general body of Pharmacology, a particular type of investigation, having as its aim the discovery of chemical substances acting specifically on pathogenic infections. This attempt to erect a new boundary fence, to delimit a new plot in the scientific field, has not passed altogether without criticism. It could be urged, and not without some justice, that the application of specific remedies to combat particular infections was as old as the use of cinchona for the cure of malarial fevers, of ipecacuanha for that of tropical dysentery, and of mercury for that of syphilis; and that the study of the action of these remedies had long been included in the orthodox pharmacology. It can even today be truly stated that, with the exact knowledge now available concerning the protozoal parasites of malaria and dysentery, chemotherapy has made no real advance on the traditional remedies for these diseases. Such improvements as have been made have resulted from a more accurate knowledge and complete separation of their alkaloids—a type of investigation having certainly a closer relation to the conventional pharmacology than to the special methods of chemotherapy. On the other hand there was justification for Ehrlich's suggestion that pharmacology had devoted attention almost exclusively to a detailed analysis of the symptoms produced in higher animals by toxic doses, even of remedies, such as quinine, which owed their therapeutic reputation to specific cure of definite infections. Chemotherapy, as he conceived it, was to shift the focus of interest to the action on the parasites. Its aim must be the discovery of substances maximally toxic for the infecting parasite, and minimally toxic for the infected host. His lifelong study of the distribution of chemical substances, especially of dyestuffs, among the organs of the body into which they had been introduced, and the chemical terms in which he was accustomed to interpret such observations, provided a conception, diagrammatic in its simplicity, for the

mode of action of such substances. The substance, given the requisite toxic properties, would kill or injure only the cells to which it became fixed by reason of its chemical affinities. The aim of chemotherapeutic investigation, therefore, must be to find toxic substances which, having a strong affinity for the protoplasm of the parasite and a weak affinity for that of the cells of the host, could be administered in sufficient doses to kill the infecting organism and leave the host unscathed. The search must be for substances which are maximally "parasitotropic" and minimally "organotropic."

It will be found difficult, as yet, to form a just opinion as to the rôle which this simple conception has played in the development of chemotherapy during recent years. It is hardly likely that it will retain permanent status as an exact scientific theory. The knowledge yet available concerning the chemistry of the protoplasm has no point of contact with a conception of this kind. Such knowledge affords no suggestion as to the nature of the chemical differences between the protoplasm of the vertebrate and that of the unicellular parasite, and furnishes no basis for prediction, or even for surmise, with regard to their differential affinities for chemical substances of known constitution. When a certain substance is found to cause the disappearance of, say, a particular species of trypanosome from the blood of an infected mouse, without harming the mouse, and fails to remove a similar infection from the rat, when administered in doses which that animal can tolerate, these observations are taken to indicate that the ratio of its parasitotropic to its organotropic properties is more favorable in the mouse than in the rat. Essentially, however, this is a mere restatement of the observed fact, that the mouse can be cured but the rat cannot. The supposition that the result is determined by the distribution of the substance between the cells of the host and the parasites is not based on independent evidence; such evidence as exists, apart from the curative result, is, in fact, not favorable to the conception. For example, the presence in the host's blood of parasites having a preferential affinity for the toxic drug might be expected to exert an antitoxic action, by diverting its action from the tissues. Certainly if this relation were found to be the rule, it would be claimed as evidence in favor of the conception. The relation actually observed, however, is the opposite of this; it is the infected animal which is the less resistant. In animals of the same species, again, suffering from the same infection, the effect of a drug on the parasites should, on the simple distribution theory, become stronger in proportion as its action

on the host becomes weaker. In some cases where a relation between the intensities of the two effects, on the infecting parasite and on the host, has been observed, it is the reverse of this; the more sensitive individual is the more easily cured. Again, the observation that, in many cases, a remedy acting potently against a certain infection *in vivo* is practically without visible action on the parasites *in vitro*, is not, by itself, consistent with the simple conception that its curative action is due to its chemical affinity for the parasites. It is true that these various anomalies can be, and have been more or less successfully reconciled with the original theory attributing curative action to specific chemical affinity; but this can only be effected by the introduction of subsidiary hypotheses, which mostly involve considerations of the interaction between the drug and the tissues of the host. The original conception can thus be redeemed from inconsistency with the observed facts; but the possibility only suffices to show that it is not necessarily untrue. Positive, independent support for the basic assumption of preferential affinity is curiously difficult to find.

In these circumstances it is impossible to feel confidence as to the general and permanent validity of Ehrlich's method of interpreting the facts. On the other hand it cannot be doubted that his bold conception has played a part of enormous importance in stimulating investigation. If the truth of a theory could be judged by the practical results which have resulted from efforts to test and apply it, this one would indeed be firmly established. But though, in the process of opening up a new territory for research, the practical value of a theory may largely be determined by its power of stimulating and encouraging experiment, and even of ignoring difficulties which a more fundamental consideration of the problem might present as insuperable, there comes a time when it is necessary to enquire whether it has not served its purpose. In the case under consideration, Ehrlich's theory has tended to focus attention on the effect of a remedy on the parasite. It led undoubtedly to the exploitation of new methods, which made a much needed departure from those of pharmacological orthodoxy. But there are growing indications that the attempt to interpret the experimental results too exclusively in terms of direct parasitocidal action may eventually hamper progress, by throwing back investigation into an empiricism from which it was Ehrlich's aim to rescue it. The object of this review is to survey the position as it presents itself to the writer at the moment, and to consider, in particular, certain facts which are difficult to reconcile with the simple distribution hypothesis. No

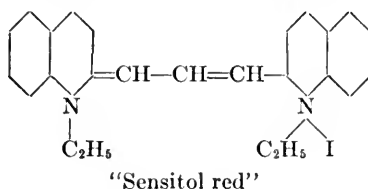
attempt will be made to mention, even by reference, the whole of the enormous wealth of detailed evidence. The aim will rather be so to present the salient and significant points as to show what part of the evidence is in conformity with the idea of direct and simple chemical action, and what part of it, on the other hand, indicates actions of much greater complexity, involving factors for the investigation of which the methods, even, are not yet available.

II. CHEMOTHERAPY OF BACTERIAL INFECTION. The attempts to combat bacterial infections by means of artificial chemical substances have been fewer in number and, on the whole, less fruitful of practical result than those directed to infections with animal parasites. Such as they are, the results are simpler and more easily interpreted in terms of direct action on the parasites. Bechhold and Ehrlich (1906, 1907), prepared a large series of phenol derivatives, introducing halogens and uniting phenolic groupings by bridges of various types, and in this way obtained compounds which far exceeded all previously known organic disinfectants in their lethal action on diphtheria bacilli growing in nutritive bouillon. The experiments were later extended to other bacilli and cocci. In therapeutic experiments, however, conducted on infected animals, none of these substances proved successful. The reason of their failure was more obvious when it was found that their disinfecting potency was enormously reduced by the presence of protein in the medium, as when the organisms were suspended for the test in blood serum instead of broth. Bechhold subsequently showed, by ultrafiltration, that the disinfectant had entered into combination with the proteins of the suspending medium, and had thus been prevented from reaching the bacteria. The observation is chiefly interesting, from its obvious significance in connection with the development of the theory of chemotherapeutic action. Further investigations by Bechhold (1909), concerned solely with disinfection outside the body, are of interest as showing a partial specificity of certain derivatives for certain organisms; thus, in the series formed by introducing successive halogen atoms into  $\beta$ . Naphthol, the optima for disinfecting action occur at different points for different bacterial species. Tribrom  $\beta$ . Naphthol, with a very high disinfecting power for pyogenic cocci and the diphtheria bacillus, had little for *B. pyocyaneus* and none for the tubercle bacillus.

This same specific liability of bacterial species to the disinfecting, or growth-inhibiting, action of chemical substances is, of course, the basis of the numerous differential or enriching media, in which the selection of one species, occurring in a mixture of many, is effected by

adding some substance—e.g., a dye, or a tellurium compound—which inhibits the growth of other organisms much more effectively than that of the one which is sought. The same selective action is clearly apparent in the results published, in recent years, by Browning, Cohen, Gaunt and Gulbransen (1922), and by Fairbrother and Renshaw (1922), on the relative disinfectant action of various dyes and related compounds on different bacteria.

These selective actions *in vitro*, where there is no question of the participation of other living cells than the microorganisms under investigation, may be regarded as presenting the fundamental problem of chemotherapy in its simplest terms. Browning, Cohen and Gulbransen (1922) find, for example, that “sensitol red,” a dye used in sensitising photographic plates to red rays, and having the constitution



has a disinfectant action on *Staphylococcus aureus* more than 2,000 times as powerful as that which it exerts on *B. coli*, when both are in peptone water. Their figures further indicate that the presence of serum much reduces its action on the staphylococcus, but somewhat increases its action on *B. coli*. On no system of hypothetical “tropic” properties of the dye for the coccus and the bacillus, on the one hand, and the serum proteins on the other, can these observations be intelligibly expressed. They must be accepted, for the present, simply as empirical facts. Yet the conditions are extremely simple compared with those which obtain in an experiment conducted on the living animal.

An attempt to study in partial isolation some of the factors concerned in the cure of bacterial infection by chemical agents has recently been made by Felton and Dougherty (1922 b). These authors measured, in a series of dyes, and in derivatives of the cinchona group of alkaloids, the toxicity for mice, the bactericidal action on *Staphylococcus aureus* in whole blood, and the inhibitory effect on the phagocytosis of staphylococci by leucocytes in serum. In a large series of dyes—in most of the triphenyl methane dyes tested, in safranines, phenazines and quinones,—and in that of the cinchona derivatives, they found that the inhibi-

tory action on leucocytes was exhibited in weaker dilutions than the bactericidal action. So far as these measurements can be taken to cover the factors at work in disinfection *in vivo*, they would be unfavorable to the efficacy of these substances under such conditions. A more favorable relation of bactericidal to leucotoxic properties was found in the case of some triphenylmethane dyes—p. Methoxy malachite green and ethyl violet—and in that of “proflavine” (diamino acridine), which, with the corresponding methyl ammonium derivative (“trypraflavine” or “acriflavine”), had already been found by Browning and his co-workers (1917, 1918) to have a powerful anti-bacterial action, augmented by the presence of serum, with a relatively low toxicity for the whole mammal or for its leucocytes. Another member of the same group, 2 ethoxy 6, 9 diamino acridine, was found by Morgenroth, Schnitzer and Rosenberg (1921) to give a very favorable ratio between disinfectant actions as determined, on the one hand, *in vitro*, and on the other in the tissues of a mouse, by local injection. Its hydrochloride has been introduced to commerce as “Rivanol,” and has been used, by local injection, to treat cellulitis and erysipelas (Rosenstein, 1921). In spite of these favorable indications there is little, if any, evidence yet available to show that these acridine dyes can cure a bacterial septicemia. Their chief practical application has been in the local treatment of infected wounds or mucous surfaces. The action of trypraflavine on trypanosome infections will be mentioned later.

The nearest approach to the successful treatment of a bacterial septicemia by a chemical agent is found in the use, by Morgenroth (1911, 1912), of an artificial member of the cinchona series of alkaloids, ethylhydrocupreine (“optochin”), in pneumococcus septicemia. Since the alkaloid has very powerful and strongly specific inhibitory and lethal effects on pneumococci *in vitro*, its action in the infected living animal seems, at first sight, a peculiarly clear example of the action of a drug directly harmful to the parasite. Even in this case, however, there is suggestive evidence in favor of the view that the immune reaction of the host plays an important part in the cure of an infection. Thus Neufeld and Engwer (1912), Engwer (1913), and especially Moore have demonstrated the enhancement of the curative effect of a specific antipneumococcal serum by doses of optochin ineffective by themselves. The enhancement is much too great to be accounted for by a mere summation, and suggests that, short of actually killing the organisms, either the chemical or immunological antagonist may so alter them as greatly to weaken their resistance to the other.

Similarly incompatible with the conception of the action of such substances, on septicemia in animals, as a direct disinfection, was the phenomenon described by Felton and Dougherty (1922 a), working with some members of the large series of artificial derivatives of cinchona alkaloids produced by Jacobs and Heidelberger (1919 a, 1920, 1922). Felton and Dougherty found an optimum dose for the prevention of septicemia, with simultaneous injection of the drug and various multiples of the lethally infecting dose of pneumococci. If the dose of the alkaloid was increased beyond that optimum, but still well below the limit of the host's tolerance, the number of lethal infective doses which it would antagonize rapidly fell again, a number of pneumococci, which a smaller dose of the alkaloid completely suppressed, now producing a spreading infection and fatal septicemia. The authors regard this as showing that there is a reversal of relationship from bacteriotropism with the small doses to organotropism with the large. One could hardly find a better example of the desperate expedients which are necessitated by the effort to make the facts of therapeutic action fit into the rigid frame of the distribution-hypothesis. When once the coöperation of the host's defensive reaction is recognized as necessary, in accordance with the above-mentioned evidence, produced by Neufeld and Engwer and by Moore in the case of optochin, the necessity for such strained assumptions vanishes. With the lower doses, the direct antibacterial action is reinforced by the host's defensive reaction, the total effect, on the analogy of the action of optochin, being much more than a mere summation. With the higher doses, even within the tolerance of the healthy animal, the defensive reaction is impaired and suppressed, and the maximum direct antibacterial action obtainable with the alkaloid *in vivo* is inadequate, without this reinforcement, to deal with the infection.

Mention should also be made of another direction in which chemotherapeutic investigation has made contact with the treatment of bacterial infections. Chaulmoogra oil had a traditional reputation in the treatment of leprosy. The treatment was made more effective by the introduction by Heiser (1914), and improvement by Rogers (1916) of parenteral injections of the oil, or of soaps made from certain fractions (Rogers). Recently a further improvement (Macdonald and Dean, 1921) has been made by the use of artificial ethyl-esters of the separated chaulmoogric and hydnocarpic acids—peculiar fatty acids occurring in the oil, and shown by Power to be characterized by the presence of a closed carbon ring. A scientific, chemotherapeutic

basis seems to have been given to this treatment by the work of Walker and Sweeney (1920), who find that soaps of these acids, while they are harmless to the ordinary bacteria, have a pronounced antiseptic or inhibitory action on cultures of all "acid-fast" bacteria. This suggests a direct, toxic action of these fatty acids on such organisms, presumably associated with their possession of a waxy envelope. In view of somewhat similar, albeit less convincing, therapeutic claims made for the soaps of other oils, e.g., cod liver oil, which Walker and Sweeney found inert in their cultural experiments, the position cannot be said to be perfectly clear; but their results are at least highly suggestive. The difficulty of drawing any certain conclusions from a coincidence between inhibiting action *in vitro* and curative action *in vivo* is illustrated by Lewis's (1917) work on the tubercle bacillus, in which a very wide survey of dyes resulted in the discovery of several which selectively stained the bacilli, and strongly inhibited growth in culture, but produced no definite curative effects.

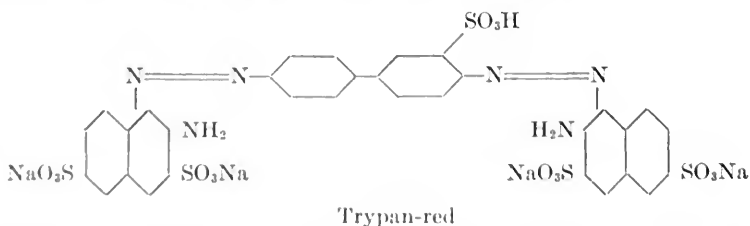
Another point of some theoretical importance, which emerges from the experiments on chemotherapy of bacterial infections, concerns the production of resistant strains. Morgenroth and Kauffmann (1912), by passage through mice treated with substerilizing doses of optochin, produced strains of pneumococci which, in the mice, were abnormally resistant to the treatment by this alkaloid. The possibility of free cultivation facilitated the study of this phenomenon of the acquisition of tolerance by bacteria under the simplest conditions. Marks (1910), by patient subculture into media containing increasing strengths of arsenious acid, produced an eight-fold rise in the resistance of a strain of hog-cholera bacillus to this substance. The treatment with arsenic raised the resistance of the organism to antimony, however, in even greater proportion—about forty-fold. The change in resistance was accompanied by modifications in the morphology and the fermentative reactions of the organism. Tugendreich and Russo (1913) similarly produced tolerance of pneumococci to optochin, and Shiga (1913) accustomed the cholera vibrio to dyes by serial cultivation *in vitro*. It is not perfectly clear whether tolerance so acquired is due to selection or to direct, heritable modification of survivors, but, in either case, the possibility of its production by the direct action of the drug, without the participation of a living host, has importance in connection with phenomena to be dealt with later.

Apart from the light which it may throw on the meaning of phenomena, which are encountered in connection with the treatment of infections

by obligatory parasites, the chemotherapy of infection by the true bacteria cannot be said to have achieved, as yet, anything of really practical importance. The use of the acridine dyes (Browning) and of the higher homologues of optochin (Morgenroth, 1917), in the local treatment of infected wounds or mucous membranes must be classed with measures of external disinfections rather than with chemotherapy in the proper sense. It is to the infections due to animal parasites that we must turn for the most characteristic and successful examples of its application, both in experiment and in practice. The spirochaets, though a strong case has been made, from the independent biological point of view, for their affinity with the vegetable bacteria, from the point of view of their infective action, and the means suited to cope with it, which is our present concern, must be ranked rather with the animal parasites.

III. CHEMOTHERAPY OF TRYPANOSOME INFECTIONS. A very large part of the systematic investigation of the possibility of obtaining specific chemical remedies has been directed to the cure of infections with trypanosomes. The organisms of this genus, responsible for several well-known tropical diseases of man and lower animals, are peculiarly well adapted for this type of enquiry. They are easily transmitted to suitable laboratory animals, and, when thoroughly adapted by passage, they produce an extraordinarily uniform type of infection, with remarkably little variation in the time of its progress to a fatal issue, in the absence of curative treatment. Progress has been made along two distinct routes, and the investigation in each direction has recently reached what appears to be an important summit of practical success, though there are doubtless higher peaks for future attainment. We have, on the one hand, the line of investigation of which the starting point was the trial of various dyestuffs, and of which the most recent, and apparently the most successful development, has been the introduction of the substance which, though uncolored, has important points of structural similarity with certain dyes, and which is known, as yet, only as "Bayer 205." On the other hand we have the line of investigation which started from the observation of a partial curative action produced by arsenious acid, has passed through a series of organic arsenical derivatives, and at the time appears to have reached a relative optimum in the introduction of the substance known as "tryparsamide." It will be convenient, in the first place, to sketch the general course of progress along the two routes separately.

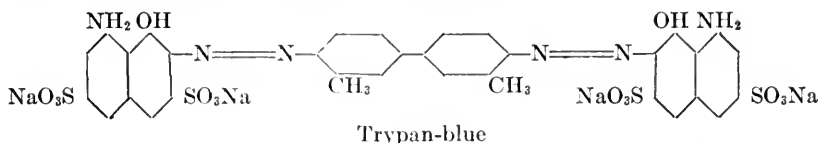
1. *The action of dyes and analogous compounds.* Ehrlich's early interest in the distribution of dyes in the organs of the living animal, and his study of their specific affinities for different cell-structures, naturally led to the attempt to discover substances of this type which, by their property of becoming selectively fixed by the parasitic protoplasm, would have a parasitocidal action in doses which the host would tolerate. The first germ of the idea is seen in his observations with Guttman, published as long ago as 1891, in which the staining of the malarial parasite by methylene blue is made the basis of an investigation of the possibility of curing a malarial infection by administering this dye. A distinct effect was obtained, but the results were not sufficiently impressive to make the dye a serious rival to quinine. Later when, with Shiga, Ehrlich began a systematic investigation of dyes as curative agents, he had at his disposal the technique of transmitting a trypanosome infection through a series of mice, as developed by Laveran and Mesnil (1902). After preliminary trials of several members of the benzopurpurin series of dyes, chosen, apparently, chiefly on account of their demonstrably long persistence in the body of a mouse into which they had been injected, the substance known as Trypan-red was obtained, through the coöperation of the dye-manufacturing firm of Cassella (Ehrlich and Shiga, 1904).



This proved to have a satisfactory curative and prophylactic value for the infection of mice with the trypanosome of Mal de Caderas (*T. equinum*). It is to be noted, however, that it was relatively ineffective against the same organism when infecting the rat, and against other trypanosomes (e.g., *T. brucei*) in the mouse. It was further observed by Ehrlich and Shiga that the dye was practically innocuous to any trypanosomes, when applied to them *in vitro* even in relatively strong solutions. This chemotherapeutic paradox is one which we shall meet repeatedly. In the case of some therapeutically active substances, such as the arsenicals, we shall see that there is reasonable ground for attributing the efficacy *in vivo*, of a drug which is relatively inert *in vitro*,

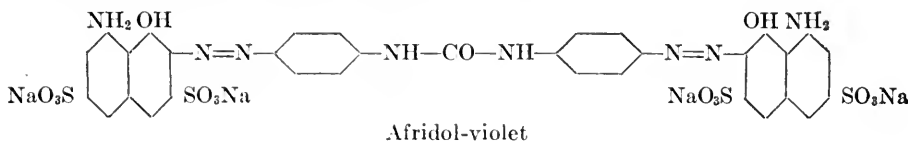
to a chemical alteration into a directly active derivative taking place in the host's body. There is no ground, save that of doubtfully justifiable analogy, for assuming that such a change takes place in the case of trypan-red, or, indeed, in that of any member of this series of curative dyes and analogous compounds.

A more practically successful member of the same group was the substance introduced into chemotherapeutic investigation by Mesnil and Nicolle (1906), and subsequently known as Trypan-blue. This substance, a derivative of tetrazotized tolidine, has an obvious similarity in its general structure to trypan-red.



It was found to be an effective curative or prophylactic agent against *T. brucei* in mice, but its practical application has been found in dealing with another type of infection, viz., that due to intracorpuseular parasites of the genus *Piroplasma* in dogs and cattle (Nuttall and Hadwen, 1909).

Another similar substance, with which promising results were obtained by Mesnil and Nicolle, was prepared by condensing tetrazotized diamino-diphenyl urea with naphthylamin-disulphonic acid.



This dye, "afridol violet," has not itself been practically applied; its greatest interest to-day lies in the fact that it presents a point of structure, viz., the presence of an amide or urea linkage, which entitles it to be regarded as the forerunner of a series of compounds prepared in recent years by the Bayer Company, one of which, known as yet only by its serial number, "205," shows promise of much greater importance than any of the immense number of dyes which have been tested for therapeutic properties. The exact constitution of "205" is still kept secret, but it is a colorless substance, with properties which indicate a high probability, as pointed out by H. King, that it belongs to a series, patented by the Bayer Company, and having a formula of the general type:



tinued presence of the substance in the body, however, is not by itself sufficient to account for the curative effect or for the artificial insusceptibility to infection, since evidence of direct lethal action on the parasites is wanting in this case, as in that of the dyes. Recourse must, therefore, be had to one or more hypotheses; but these, since they have as yet received very little support from independent evidence, cannot be regarded as much more than convenient resting-places for the mind. We may suppose, for example, that the drug only produces its effect by long-continued action, and that the relatively brief period during which its action can be observed under the microscope does not suffice for it to become visible. If that were the case an important factor in the curative efficacy would be the property of becoming fixed to the host's tissues, and liberated therefrom into the body fluids in small concentration, but over an extended period. Or it may be suggested, as has been done, that the essential curative action depends not on the direct killing of the parasites, as observed by their loss of motility and rapid degeneration under the microscope, but on destruction of their power of multiplication. This is in conformity with the observation that trypanosomes treated with a dye outside the body may lose their infectivity without visible changes in their motility or their structure. It is stated also (Busck), that trypan red will stop the multiplication of free-swimming ciliates without impairment of their other functions. We shall meet this suggestion in other connections, but the positive evidence in its favor is hardly sufficient to warrant its adoption as a general chemotherapeutic principle, or its facile invocation to explain any discrepancy between actions outside and inside the body. If it is really the secret of the paradox which immediately concerns us, it is obvious that fixation by the host's tissues, and the resulting mild, persistent action on the parasites of the gradually liberated drug, must be a factor in the effect. Another possibility is that the curative substance is not directly hurtful to the parasites, but that some parasitocidal derivative is formed from it by interaction with the host's tissues. We shall find that there is evidence in favor of such a view in the case of certain arsenical compounds, from which derivatives having a powerful, directly lethal action *in vitro* can be produced. No such evidence is available in the case of the substances which we are now considering, or indeed in that of any of the curative dyes or non-metallic compounds; the suggestion is possible, but no more. The same is true of another suggestion, namely, that the direct action on the parasites may not be due either to the substance as

administered, or to any derivative therefrom, but to something produced by the tissues of the hosts in response to an action on their cells. Again, there is no evidence in favor of such a view: it is simply a convenient assumption to explain a difficulty.

It will be clear that all these possibilities, and indeed any which can be invoked to explain the curative effect in the host, of substances not obviously hurtful to the parasites outside the body, almost of necessity involve a participation of the host's organs in the curative action. Dyes were made the starting point of this line of investigation, because of the ease with which their distribution between tissues and parasites could be followed by the eye. Yet in this group the evidence so obtained is in favor of a strong affinity for the host's tissues rather than for the protoplasm of the parasites; these dyes can be seen to be strongly "organotropic," while the evidence for their directly "parasitotropic" properties is, at best, not strong.

If we take the known facts concerning the action of such a substance as "205," without reference to any particular theoretical conception, we can say that this substance, having no demonstrable direct effect on the parasites, causes their complete disappearance from the body of an animal infected with them; that for a long period after a single injection of "205" the animal is resistant to further infection by trypanosomes, and that during that period its body fluids contain something which possesses the property of curing infection; but that there is no clear evidence as to whether that something is a remnant of "205" itself, or some derivative thereof, or some antagonist produced by the host itself in response to the stimulation of its tissues by "205." The analogy of the dyes, with their demonstrable long persistence in the host's tissues, suggests, but by no means proves, that "205" is itself the substance at work.

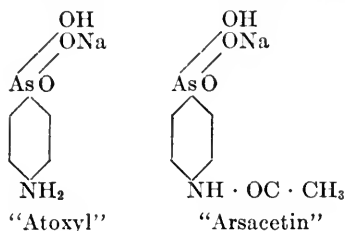
The successful experiments on the cure of infected animals by "205" have been followed by a series of apparently complete cures of well-advanced infection in man with *T. gambiense* and *rhodesiense*. A single injection has not proved adequate for the treatment of the human patient, but a course of eight or more injections has succeeded in producing what appear to be permanent cures, in a number of cases which, a short while ago, would have been regarded as quite beyond the reach of any treatment. The toxic effect of the drug itself on such patients has been a temporary and apparently not serious nephritis. (Mühlens and Menk (1921), Yorke (1921), Low and Manson-Bahr (1923).)

Another group of dyes of which the curative action in trypanosome infections has been investigated is that of the triphenyl methane dyes.

The results with these have been, on the whole, of theoretical rather than practical importance. Wendelstadt and Fellmer (1906) experimented with Malachite Green, and obtained evidence of curative action, but associated with so much local injury of the host's tissues as to make its practical application impossible. Ehrlich and his co-workers tried a number of dyes of this group and obtained promising results with Parafuchsine, though the local action on the tissues was still undesirably powerful with this substance. A chlorinated derivative of Parafuchsine, called "Tryparosan" (Roehl, 1909), was found to be more powerfully curative and at the same time much less toxic.

Another series of dyes examined by Ehrlich (1909a) and his co-workers was that containing pyronine, acridine derivatives, and related oxazines and selenazines. Of these the substance first known as "Trypaflavin" (diamino-methyl-acridinium ehloride) gave curative results in mice infected with trypanosomes which, in view of its low toxicity, seemed highly promising of practical value. Its main practical use, however, has proved to be as a bacterial antiseptic, and the importance of this group of dyes, as a whole, is due more to the highly interesting results which they have given in connection with the production of tolerant strains, considered in a later section, than to their practical therapeutic value.

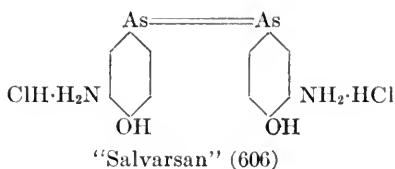
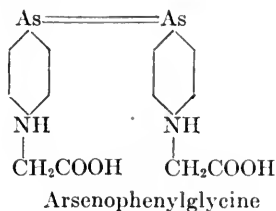
2. *The action of compounds containing arsenic.* Lingard (1899) had treated Surra in horses successfully with arsenious acid, but Laveran and Mesnil (1902), confirming Bruce's (1897) earlier observations in Africa, found that it produced only a temporary disappearance from the peripheral blood of the trypanosomes of Nagana (*T. brucei*). The application, by Thomas and Breinl (1905), of the organic arsenical derivative, which had for some time been used in treating skin diseases under the name "atoxyl," registered a definite advance. Its efficacy was soon confirmed by a number of workers, but the next step of importance was the elucidation by Ehrlich and Bertheim (1907) of the true structure of "Atoxyl" which, since it proved to be a para-amino phenyl arsenic acid, with a reactive free amino-group, formed a favorable starting point for a series of derivatives.



The first of these derivatives showing a more favorable experimental ratio between the tolerated dose and the dose curative of trypanosome infection, was produced by acetylating the amino-group, and named "Arsacetin." Neither Atoxyl nor Arsacetin has fully justified the early hopes of its value as a remedy in human trypanosomiasis, both having been found, in practice, to produce permanent blindness in a serious proportion of the patients treated. The study of their action, however, had results of great theoretical importance. Like the benzin dyes, above-mentioned, these arsenical derivatives had no action on trypanosomes *in vitro* which could explain their curative action. Uhlenhuth, Hubener and Woihte (1908) reject the view that its action in the body is due to liberation of the arsenic in inorganic form—which would, indeed, be difficult to reconcile with the comparative inefficacy of the inorganic compounds when directly injected—and attribute the curative effect to the action of Atoxyl on the body cells, causing an increased formation of protective substances. The experiments of Levaditi, Brimont and Yamanouchi (1908, 1909) gave greater precision to such a conception, and suggested a participation of Atoxyl itself or some derivative of it, in the composition of the protective substance. They found that when a solution of atoxyl is mixed with an emulsion of liver, a product is obtained which is directly toxic to trypanosomes. The possibility of precipitating this toxic substance with alcohol and its instability to heat led Levaditi (1909) to conclude that it was an arsenic-protein complex, to which he gave the name "trypanotoxyl." He regarded the mechanism as a reduction of Atoxyl by the liver substance, and a combination of reduction-product with protein, the latter acting as the link for anchorage to the trypanosome. Ehrlich and Roehl (1909 b, c), however, had already shown that reduction of Atoxyl, by any ordinary reducing agent, produced, in para-amino phenyl arsenious oxide, a substance having a very intense direct toxicity for trypanosomes. According to Roehl's experiments, the function of the liver emulsion in Levaditi's was simply to reduce Atoxyl to arsenious acid, the subsequent combination of this with protein merely weakening its action. On the whole the weight of evidence seems in favor of this simpler view, that the activity of Atoxyl in the body is due to its reduction to the arsenious form, with a trivalent arsenic, by the action of the tissues. The reduced product is not only toxic for trypanosomes, but much more toxic than Atoxyl for the host; and it was in harmony with Ehrlich's view, that mice which were easily poisoned by Atoxyl were likewise more easily freed by it from trypano-

some infections than those which were more resistant to its action. Reference must be made later to some difficulties arising in connection with the tolerant strains. Meanwhile, we may note that Ehrlich's view of the action of Atoxyl departs from his original chemotherapeutic conception, in that the efficacy of Atoxyl is attributed to an immediately organotropic property. It is the reduced arsenious compound, formed in virtue of the original organotropism, which is now held to be parasitotropic.

The point of greatest importance, however, was the establishment of the superior curative activity of the compound with trivalent as compared with that containing pentavalent arsenic. This led to the production of a series of compounds containing trivalent arsenic, of which the important ones were arsenophenylglycine and dihydroxy-diamino-arsenobenzene, which, in the form of its hydrochloride, has achieved world-wide celebrity under the serial number "606," and the names "Salvarsan," Arsenobenzene, Arsphenamine, etc.



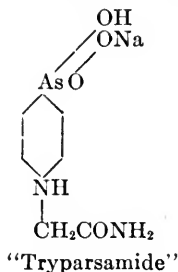
Both these substances are reduced beyond the arsenious-oxide to the arseno-condition, with a double molecule, formed by the linkage of the free valencies of the two arsenic atoms. Arsenophenylglycine gave highly promising results as a curative agent in experimental trypanosomiasis, but it failed to fulfil this promise when applied to the treatment of sleeping-sickness in man. In the case of salvarsan, which was produced by Ehrlich and Bertheim (1912), in the course of Ehrlich and Hata's (1910) search for a remedy for spirochaetal infections, the powerful curative action in trypanosome infections has been somewhat overshadowed by the rapid and successful development of its use in the spirochaetal infections. Here we may note that, just as in the case of the fully oxidized derivatives of arsenic acid, we find again, in these fully reduced arseno-compounds the paradox of intense curative action on trypanosome (or spirochaet) infections *in vivo*, with no direct action *in vitro* to explain it. And again, in the case of salvarsan, we find, in the corresponding partially oxidized derivative, p-hydroxy m-amino phenyl arsenious oxide, a substance with an intense lethal action on

trypanosomes (or spirochaets) outside the body. There have again, as in the case of atoxyl, been suggestions of the formation in the body of some trypanocidal or spirocheticidal complex between the proteins of the blood or the tissue cells and salvarsan or some derivative thereof; but again the facts appear to be satisfied by the simpler theory that the parasitocidal substance is the partially oxidized arsenious-oxide derivative. This is very clearly indicated in recent papers by Voegtlin and Smith (1920) who, using a method first adopted by Morgenroth and Rosenthal (1911) for measuring the rate of disappearance of trypanosomes from the blood after curative injections, found that the effect began immediately in the case of the arsenoxid, after a definite latent period in the case of the arseno-compound, and after a longer latent period in the case of arsenic acid derivatives. These latent periods correspond with the greater liability of the arseno-compound to oxidation than of the arsenic-acid form to reduction, and the conclusion is drawn that, in either case, a preliminary to the curative effect is oxidation or reduction to the arsenoxide.

This view gives a satisfactory explanation of the therapeutic efficacy in the body of the arsenical compounds which are practically inactive on the parasites outside it. It leaves still to be explained, however, the choice for therapeutic purposes of substances which, only after injection, give rise to the directly parasitocidal derivatives, rather than these latter themselves. It is true that the arsenoxides have a much greater toxicity, not only for the parasite, but also for the host. But this alone will not explain their unsuitability for therapeutic use; for, tested on mice infected with trypanosomes, they show a high ratio between the tolerated dose and that needed to free the blood from trypanosomes, though the absolute dosage is in both cases on a lower level. The explanation must, I think, be sought by considering a factor, in the efficient treatment of infections with trypanosomes or spirochaets, which has hitherto hardly received adequate attention, namely, the factor of persistence of action. If the ready-formed arsenoxide is injected, there will momentarily be present in the blood a concentration of the direct parasiticide higher than is ever produced by its slow formation from either the more reduced or more oxidized compound; but the arsenoxide is so toxic for the host that the concentration cannot in any case be raised to a high level with safety, and the dose given at one injection must be small. To obtain the optimum effect, by administration of the arsenoxide itself, it would probably be necessary to maintain for hours or days a continuous infusion of the substance in very high dilution. This slow, persistent supply of the arsenoxide can be maintained for relatively long periods by

injecting the arsenic-acid or the arseno-derivative. In the case of salvarsan the persistence is aided by the insolubility of the free base, which must, at the reaction of the blood and tissues, be liberated and largely deposited.

This factor, of the liberation of the directly parasiticidal product of partial oxidation or reduction, is probably of importance in the action of all members of this group. And, in default of any clear knowledge of the factors which determine the rate of excretion of the parent substance, or of the rate of its change into the arsenoxide form, either of which may be affected by small changes in the nature and position of the groups attached to the benzene nucleus, there is clearly no warrant for the assumption that a change in structure, which increases the therapeutic effect, does this by increasing the preferential affinity for the protoplasm of the parasite, or by introducing an affinity for a new type of "chemoreceptor." The curative process is too complicated to be expressed in such simple terms, and involves the coöperation of the host's tissues, that is to say, some degree of "organo-tropic" property. It is highly probable that, in the treatment of different types of infection, the optimum rate and most favorable site for the production of the directly active substance, will in one case be provided by use of an arsenic acid derivative, in another by that of an arseno-derivative. Both have their special drawbacks. The arseno-derivatives are unstable, sensitive to exposure to air, and very difficult to obtain pure. Slight differences in the constitution, especially of the derivatives with sulphur-containing radicles attached to the amino-groups, may cause wide variations in toxicity and in therapeutic potency. Nevertheless, the arseno-derivatives are the most effective of the arsenical compounds yet obtained in the treatment of spirochaetal infections. On the other hand, the most successful arsenical compound yet tried in the treatment of human sleeping-sickness (due to *T. gambiense*) appears to be an arsenic-acid derivative, the substance known as "tryparsamide," an amide of the arsonic-acid compound corresponding to arsenophenyl glycine.



This substance, prepared by Jacobs and Heidelberger (1919 b, c), and selected by Brown and Pearce (1919) from a large series as the most favorable in its curative action, has now been used with highly promising results in the treatment of human sleeping-sickness. (Pearce (1921), Chesterman (1923).) It shares with "Bayer 205" the responsibility for the much more hopeful position which has recently developed with regard to the treatment of that disease. Like its parent, atoxyl, "tryparsamide" is not free from the liability to produce blindness.

3. *Antimony, bismuth and other metals.* Cushny appears to have made the first suggestion of a trial of compounds of antimony and bismuth, on account of their close chemical relationship to arsenic. Certain antimony compounds have been found effective in infections by trypanosomes, but not with equal certainty those of bismuth, while the reverse, as we shall see, is true of spirochetal infections. In neither case have the compounds used been aromatic complexes, with the metal attached to a benzene ring, as in the case of the arsenicals. The antimonyl—and bismuthyl—tartrates have presumably been chosen chiefly on account of their convenient solubility. Tartar emetic and its sodium analogue were used with success by Plimmer, with Thompson and Bateman (1908), and simultaneously by Mesnil and Brimont (1908 a). There are many later records of their experimental and clinical use. The action on the trypanosomes is apparently a simple and direct one, the parasites being killed outside the body by high dilutions of tartar emetic. (Mesnil and Brimont). Metallic antimony suspended in oil and injected intramuscularly is apparently as effective as the antimonyl tartrate (Plimmer and Bateman), and antimony trioxide is stated to be even more efficacious. (Kolle, Hartoch, Rothermundt and Schürmann, 1913.) There appears to be no need for a more complicated assumption than that of a directly lethal action on the parasites, which must be much more sensitive to the antimony compound than are the tissues of the host. The contrast between this simple type of action and that of substances like "Bayer 205" and "tryparsamide" is the more interesting in view of the fact that, up to the time of the introduction of these latter, tartar emetic and the simple compounds of trivalent antimony, such as the trioxide, were among the more effective of the therapeutic agents tried in human trypanosomiasis. It emphasizes the possibility that the special efficacy of a complex arsenical remedy, at any rate, is due rather to the favorable rate and locality of the production from it of some simpler, directly trypanocidal substance, than to any specific affinity for the protoplasm of the trypanosome due to its molecular configuration.

The bismuth analogue has been tried in experimental trypanosomiasis with only partial (Frouin and Guillaumie, 1921) or no success (Adler, 1921). We shall see, on the other hand, that it is highly efficacious as a remedy for spirochaetal infections. Frouin and Guillaumie obtained somewhat weaker actions on trypanosome infections of mice, with cerium, yttrium and rhodium salts, but none with those of niobium.

Compounds of other metals have chiefly been used for combined treatment, together with those containing arsenic or antimony. Moore, Nierenstein and Todd (1907) advocated a combined use of atoxyl and mercury salts, but Plimmer and Thomson (1908) found that, in dosage sufficient to free the blood from trypanosomes, such treatment produced fatal lesions. Loose chemical combinations of salvarsan with other metals, such as gold or copper, have been found effective in treating experimental infections with trypanosomes, but the only compound of this kind, the "silver salvarsan" to attain practical importance has found its use in the treatment of spirochaetal infections.

4. *Resistant strains.* One of the most interesting and theoretical important chapters in the chemotherapy of trypanosomiasis is that dealing with the development of resistant strains. When an inadequate dose of a curative agent has been given, so that a relapse of the infection occurs, further treatment is often found to be less effective, and the strain, transferred to another animal of the same species, retains its resistance and continues to do so through an indefinite number of passages. By carefully graded treatment it is possible to raise the resistance to a high level, at which it is maintained during further passages without treatment. (See Ehrlich (1907, and Browning (1907, 1908).) The phenomenon was first observed in the case of atoxyl, but was soon found to apply to other curative agents, such as the dyes. A strain resistant to trypan red was normally sensitive to atoxyl, and *vice versa*, but several such specific resistances could be developed in the same strain by the appropriate treatments.

The phenomenon at first seemed to give strong support to the conception of specific affinities for special groups in the protoplasm of the trypanosome. If the chemoreceptors for atoxyl disappeared, or were greatly reduced, the strain became proportionately resistant to the effect of this substance. When a strain resistant to atoxyl was found to be still sensitive to arsenophenylglycine, this indicated that the latter was attached to a separate "acetico-receptor," which the treatment with atoxyl had not removed. The problem, however, has proved

to be by no means simple. In the case of an agent acting simply and directly, like tartar emetic, it can indeed be shown that the trypanosomes of a strain resistant to tartar emetic will tolerate *in vitro* a higher concentration of the drug than the original, sensitive strain (Mesnil and Brimont, 1908 b). On the other hand the resistance to tartar emetic was best produced by treatment of the infected animal with arsenious acid, while resistance to the latter itself was only obtained by very prolonged and careful treatment (Gonder, 1912). In some cases treatment with arsacetin or arsenophenylglycine has produced strains resistant not only to these substances but to tartar emetic. A still greater complication is introduced by the behavior of a resistant strain when transferred to a host of another species. Mesnil and Brimont (1908 b) produced a strain resistant to atoxyl in the mouse, and found that, when it was transferred to the rat, the sensitiveness disappeared and the strain remained normally sensitive during 40 passages through this host-species, to regain its resistance immediately when retransferred, without further treatment, to the mouse. In the dog, on the other hand, the resistance acquired in the mouse was retained. There could be no clearer evidence of the coöperation of the host's tissues in the curative action of atoxyl; on the other hand, it becomes very difficult even to speculate on the mode of their intervention. If their action were merely to reduce the atoxyl to the arsenious condition, we should have to suppose that the resistance of the parasite was to the arsenoxide; but this gives no explanation of the fact that the resistance is a property of the strain of trypanosomes and not of the hosts. Moreover there is a perplexing observation recorded by Ehrlich (1909 a) in which a strain rendered resistant, in mice, to partially oxidized arsenophenylglycine was found to be *more* sensitive to this compound *in vitro* than the normal strain. He interprets this in accordance with his view that the lethal effect visible *in vitro* is not the same as that which is effective in the body, attributing the latter to stoppage of multiplication. But such an explanation fails entirely to touch the problem presented by a resistance exhibited in one host species and not in another. Levaditi (1909), on his theory of a complex arsenic-protein poison, would regard the resistance in the mouse as specific for mouse "trypanotoxyl," leaving the strain normal in its reaction to rat "trypanotoxyl," but again it is difficult to suppose that a specific difference, so sharp as to discriminate between mouse and rat proteins, should not exist between mouse and dog proteins. It is evident that no satisfactory solution of these difficulties is yet available, and we must be content to note the

indication which they give of the complicated nature of the chemotherapeutic process, and of the essential coöperation therein of the host's tissues.

One other highly interesting series of phenomena must receive passing mention. The resistance to drugs appeared at first to be highly specific. Later work of Ehrlich's pupils (Kudicke (1911), Gonder (1912)) showed an extraordinary reciprocal relation in the development of resistance between certain dyes, on the one hand—pyronines, acridine derivatives, oxazines, selenazines, all possessing, as pointed out by Ehrlich, an orthochinoid structure—and the arsenicals, such as atoxyl, on the other. Resistance to either of these groups seemed to confer resistance to the other; and, indeed, the most rapid and effective method of producing an atoxyl-resistant strain was found to be the injection into an infected animal of a non-sterilizing dose of a dye, such as trypanflavin. To state that the chemoreceptor for arsenic and for orthochinoid dyes must be identical, is to offer no explanation for a wholly mysterious phenomenon; it is merely to state the fact of the reciprocal action in producing resistance, in other words. Nor is it rendered, as yet, more comprehensible by the extraordinarily interesting observation of Werbitzki (1910), confirmed and extended by Gonder (1912), that trypanosomes of a strain rendered resistant to one of these dyes, though otherwise apparently normal, have lost the kinetonucleus or blepharoplast, which, according to Laveran and Roudsky (1911), can be seen to become selectively stained, and then to disintegrate under the action of the dye. Resistance to arsenicals, produced directly by ineffective treatment with these, has no effect on the morphology of the trypanosomes.

IV. SPIROCHAETAL INFECTIONS. Interest in the chemotherapy of these has, until recently, centered almost wholly round salvarsan and its soluble derivatives (neosalvarsan, sulfarsenol, sulpharsphenamine, etc.). It is beyond the purpose of this review to discuss the enormous literature on their action. We may be content to note the greater efficacy of the original "606," thrown out of true solution at the reaction of the blood, as compared with that of the derivatives soluble at neutral reaction, when both are given intravenously. It may be suggested that the factor of persistence of action is here at work, the active arsenoxide being slowly liberated over a long period from the insoluble base deposited in the tissues; and this suggestion receives support from the observation that the more soluble and readily excreted derivatives acquire an enhanced efficacy with hypodermic

injection. Some of these preparations, indeed, owe their popularity to the possibility of giving them hypodermically without causing excessive pain and induration. The increased curative action obtained by associating a silver compound with salvarsan, producing the so-called silver-salvarsan, is not yet adequately understood. It was supposed that a silver salt (nitrate or sulphate) formed an addition-compound with salvarsan, the silver and arsenic combining in virtue of residual affinities. Recent work seems to have supported the simpler, if less precise, conception, that the compound consists of silver chloride or oxide in colloidal solution, protected from aggregation by the emulsoid salvarsan. It must be left an open question whether the silver as such participates in the spirocheticidal action, or whether its association with the salvarsan favors the liberation of the directly therapeutic arsenoxide in the body.

Of fresher interest is the experimental demonstration by Levaditi and Sazerac (1921, 1922), of the curative action in syphilis, and in the closely analogous natural infection of rabbits with *Spirochaeta coniculi*, of the bismuthyl tartrate of potassium and sodium—a bismuth analogue of tartar emetic. Fournier and Guenot (1921), (1922) have used it extensively and with success in treating human syphilis. The sodium salt had already been used by Robert and Sauton (1916) for experimental treatment of spirochetosis in fowls. It will be noted that again, when arsenic is replaced by one of its chemical relatives, antimony or bismuth, the necessity for employing a complex and unstable organic derivative seems to disappear, and a relatively simple salt of convenient solubility seems to have an adequate therapeutic effect. Later experience has tended to emphasize the view that it is bismuth, administered in any form which will secure adequate absorption, which is the essential chemotherapeutic agent in this case. On the other hand, in the first observation of the action of bismuth compounds on a spirochaetal infection, in which Robert and Sauton demonstrated its curative effect in infections with *Sp. gallinarum*, these authors record that the sodium bismuthyl tartrate was without action on the parasites *in vitro*.

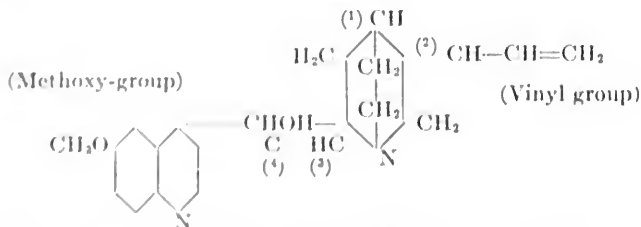
Recently Fournier, Levaditi and Schwartz (1922) have examined compounds of niobium, tantalum and vanadium. The last alone showed any effect on experimental or clinical syphilis, but these effects of vanadium were comparable to those obtained with the analogous bismuth salts. Pröschner, Seil and Stillians had already (1917) shown the effect of vanadium on syphilis.

We may note that, in their long series of experiments, which led ultimately to salvarsan, Ehrlich and Hata tried a number of dyes in spirochetal infections, without any results of value. "Bayer 205" which, though not a dye itself, may be regarded as the most successful achievement, up to the present, of chemotherapeutic investigation on compounds of this type, appears to be without effect on spirochetal infections. Up to a certain point, and especially in connection with the arsenical remedies, trypanosomes and spirochaets appear to show a certain community of response to therapeutic agents; thereafter divergence is apparent, the lines of investigation, which at the moment show the greatest promise for new development, being, in the one case, in the direction of complex organic substances related to the dyes, in the other case, in the direction of relatively simple salts of the metals related to arsenic and antimony.

V. LEISHMANIASIS. The successful treatment of *Leishmania* infections has hitherto been practically limited to the intravenous injection of tartar emetic and its sodium analogue. The use of these salts appears to have resulted from empirical trial by different workers (Vianna (1913), Carini (1914), Cristina and Caronia (1915), Rogers (1915)), working merely on the broad analogy of its previous use in other protozoal infections. It was remarkably successful, though the arsenical remedies had been relatively valueless. At the time of writing the first news is published of the successful treatment with "Bayer 205" of a case of kala-azar, which had resisted treatment by tartar emetic (Yorke, 1923). Simultaneously there is published by Lindenberg (1923) an account of the treatment by "Bayer 205" of the cutaneous Leishmaniasis of Brazil. Cases can apparently be cured by hypodermic injection, but local application of "205" to the sore is without effect: a most significant observation, if confirmed. This is but one example, out of a number, which will present themselves, of the lack of correspondence between the facts of chemotherapy and the accepted zoölogical affinities. "Bayer 205" is highly effective in certain trypanosome infections, quite ineffective in others, but apparently effective again in Leishmaniasis. The *Leishmania* parasite has, indeed, some zoölogical affinity with the trypanosomes, but hardly so close as that of different species of trypanosome for one another. We may note, in the same connection, the common liability of trypanosomes and spirochaets to the action of arsenical remedies such as salvarsan, which *Leishmania* does not fully share. No theory as to the systematic position of the spirochaets can suggest for them a closer biological affinity to the trypanosomes than that of *Leishmania*.

VI. MALARIA. For the treatment of malaria the modern development of chemotherapy has effected practically nothing. Since cinchona bark was brought to Europe in the 17th century, real improvements in the treatment of malaria have been almost limited to improvements in the administration of this drug and its alkaloids, guided in the more recent period by the recognition of the malarial parasites and more accurate knowledge of the chemistry of the cinchona alkaloids. Ehrlich and Guttman's (1891) trial and recommendation of methylene blue has been mentioned. Later observations have not supported its claim to rivalry with quinine, nor have any of the newer remedies for other protozoal infections, such as salvarsan, acquired an importance in the treatment of malaria at all comparable with that of quinine. The special reputation of quinine, as compared with the other naturally occurring cinchona alkaloids would appear to be largely artificial. It apparently arose at a time when quinine and cinchonine were the well-known and characterized alkaloids, the latter contrasting unfavorably with quinine on account of its toxicity for man. In recent years, with improving knowledge of the cinchona alkaloids, others have been tried, with results which seem to indicate that some of them—hydroquinine, quinidine, cinchonidine—are at least as effective in the treatment of malaria, and not noticeably more toxic. (McGilchrist (1915), Giemsa and Werner (1914), Acton (1920).)

The point has practical importance since the whole policy of the artificial cultivation of cinchona has hitherto been governed, both in selection of soil and climate, and the selective breeding of the trees, by the supposed necessity of securing a maximum yield of quinine in proportion to the other alkaloids. Theoretically it is interesting as showing that neither the vinyl group, which is reduced to an ethyl group in hydroquinine, nor the methoxy-group, which is absent in cinchonidine, nor the precise stereometric configuration of the quinine molecule, since quinidine differs in its arrangement in relation to at least one of the four asymmetric carbon atoms, is essential to the therapeutic action of quinine in malaria.



Quinine. (1), (2), (3) and (4) are the 4 asymmetric carbon atoms.

With the exception of hydroquinine, none of the many artificial derivatives which have now been made from these cinchona alkaloids have proved to be even as good as the natural ones in treating malaria: they have found their practical uses in other directions, in the treatment of bacterial infections. The main question of chemotherapeutic interest, therefore, is still that of the manner in which quinine and its similarly acting relatives produce their antimalarial effect. It is a curious item in the history of the subject that Binz, as long ago as 1867, should have correctly predicted that the cause of malaria would prove to be a protozoön, on the quite inadequate ground of his observation that quinine, with its traditional value in malaria, had a lethal action on free-living protozoa (paramecium etc.) in relatively high dilutions. It would have been as logical, from the failure of quinine to cure tropical dysentery or sleeping sickness, to conclude that the infective agents in these diseases were probably *not* protozoa. After the discovery of the malarial plasmodia various attempts were made to observe the effect of quinine on the parasites. Many such observations were directed to following the degenerative changes in the parasites during the treatment of the patient with quinine; these, of course, leave quite open the mode of action of the remedy, whether directly on the parasite, or indirectly, by stimulating the defensive response to the host. Observations on the effect of quinine added to malarial blood *in vitro* have not produced any conclusive evidence in favor of a direct parasitocidal action of the alkaloid. (Laveran (1891), Dock (1891), Marchiafava and Celli (1886), LoMonaco and Panichi (1900).) The last named authors appeared to regard the effect of quinine, in medicinal doses, as a stimulation of the young parasites, leading them to leave the red corpuscles and so to become exposed to the unfavorable conditions of the blood-plasma. This observation has an obvious relation to the interesting theory of quinine action much more recently put forward by Morgenroth (1918). This observer, as the result of tests made by physiological and bacteriological methods, came to the conclusion that, when quinine is added to shed blood, a very high percentage of it becomes extracted by the corpuscles, the serum or plasma being left almost free from quinine. On the basis of these observations Morgenroth puts forward the suggestion that quinine owes its curative action in malaria to its *organotropic* properties, especially its affinity for the red corpuscles. He leaves it an open question whether the quinine, so located, acts in relatively concentrated form on the already intracorpuseular parasites, or whether, as

he thinks more probable, the quinine, being concentrated on, or in, the surface layer of the corpuscle, repels the merozoites resulting from the asexual reproductive cycle, so that, being denied access to the place of their further growth, they perish in the plasma. While it is of interest to find Ehrlich's former colleague thus frankly adopting a view, which attributes the curative action of quinine to an organotropic and not a parasitotropic property, it must be borne in mind that none of the numerous determinations, of the distribution of quinine between corpuscles and plasma, which have been made by chemical means, give any support to Morgenroth's deduction from his biological tests. Ramsden, Lipkin and Whitley (1918) found a ration of 2 or 3 to 1 in favor of the concentration in the plasma, while later determinations, by Acton and King (1921), show a practically equal distribution throughout the blood mass. The discrepancy is obviously one which demands further investigation. For our present purpose it is sufficient to note that no simple conception, of directly lethal action on the malarial parasites, will fit the knowledge yet available as to the nature of the curative action of quinine in malaria.

VII. AMOEBIASIS. As in the case of malaria, the only drug which, even to-day, can be stated to have an indisputable and specific action on tropical dysentery, was introduced to Europe from South America in the 17th century for the treatment of that disease, more than two centuries before the organism responsible for it was discovered. The alkaloids of ipecacuanha root like those of Cinchona bark, have now been isolated and characterized; and again we find among them, in emetine and cephaeline, principles having an antiamebic action more potent and specific than that of any other known substance, including such artificial derivatives of emetine as have hitherto been prepared. The *Entamoeba histolytica* is an organism much more readily accessible for direct observation of effects *in vitro* than the malaria parasite; and until a few years ago the evidence seemed to point distinctly to the simplest possible mechanism for the curative action of emetine in amebic infections, namely, a directly lethal action on the amebae. Vedder (1911) showed that the alkaloids, in relatively high concentration (1 in 100,000) would kill free-living amebae, though Lyons (1912), who made similar experiments, was doubtful as to the adequacy of the results to explain the therapeutic action. Rogers' (1912) experiment, which indicated that *Entamoeba histolytica*, fresh from the human intestine, was killed in a few minutes by emetine in a dilution of 1 in 100,000, suggested that there was no need to look further than simple,

direct action on the parasite for an explanation of the therapeutic action in the patient.

Repetition of such observations, and their extension to the other alkaloids of ipecacuanha, to artificial derivatives therefrom, and to other substances, have made this simple view quite untenable. Kuenen and Swellengrebel (1913) observed a lethal action of emetine on *E. histolytica*, but it needed from 1 in 10,000 to 1 in 5,000 to produce the effect, and periods up to many hours for its development. The experiments of Kolmer and Smith (1916), and of Walters, Baker and Koch (1917) also showed a much lower order of activity for emetine on parasitic or free-living amebae. Dale and Dobell (1917) studied the effects of a number of these alkaloids on the fresh entamebae in the test-tube, obtaining the material mostly from cats infected from human cases of dysentery. Some of the same substances have been clinically tested on man by G. C. Low. A very curious contrast is revealed. While Dale and Dobell did not find emetine without effect on the isolated amebae, both it and cephaeline were far less active than Rogers' statement had suggested, and less so than other alkaloids, such as quinine and harmaline, which have no curative action in an amoebic infection. This has, in essence, been recently confirmed by Allen (1920), using amebae direct from human patients. On the other hand, one of the minor natural alkaloids from ipecacuanha, methylpsychotrine, and several artificial derivatives, such as demethoxyemetine, were found by Dale and Dobell to exhibit a more powerful toxic action for the isolated amebae and a relatively very low toxicity for the mammal. These, when tested on human sufferers from dysentery, all failed entirely to justify this promise of therapeutic value; so far as results at present available warrant a conclusion, it would be that the therapeutic efficacy of these alkaloids, in dysentery, has no relation to their toxicity for *Entamoeba histolytica* outside the body, but is, roughly, in direct proportion to their toxicities in man.

Another curious fact which emerged was that a strain of *Entamoeba* which was readily susceptible to treatment by emetine in the human patient, was completely resistant to emetine in kittens, to which it had been transferred from man before the treatment was applied. The close connection of the curative action of emetine with the behavior of the organism as a tissue-parasite is made clear by the fact that treatment with emetine eliminates infection with *E. histolytica* and leaves undisturbed the saprophytic entamebae and allied forms (*Entameba coli*, *Endolimax nana*) living in the contents of the colon. We are,

once more, almost compelled to suppose that the tissues of the host play an essential part in the curative action which the drug initiates. It is, of course, just conceivable that emetine, while not visibly affecting the motility and vitality of the amebae, when applied in high dilutions, has nevertheless a powerful inhibiting action on their reproductive activity, which, since artificial culture has not proved possible, cannot be detected outside the body. So far as experiments on free-living amebae in culture can provide sound evidence on this point, it is not in favor of such a view. Pyman and Wenyon (1917) found, indeed, an inhibiting action produced by emetine and cephaeline and none by psychotrine, which has no curative action. They observed, however, equally good inhibition of multiplication by *N. methylemetine*, the curative effect of which is, at best, very slight. It would be rather a forced assumption, moreover, to suppose that alkaloids nearly related to emetine, and having a more pronounced immediate and visible action, should be devoid of the inhibiting action on reproduction; and the assumption could not, in any case, explain why emetine inhibits the reproduction of the entameba in man, but not that of the same strain in the cat. To suggest, as an explanation, coöperation of the host's tissues, in a manner concerning which the knowledge yet available affords not even a hint, is unsatisfactory; but it seems at present the only alternative to a completely agnostic attitude.

VIII. HELMINTHIASIS. Strictly speaking we ought to include under the heading of "chemotherapy" the removal of parasitic worms from the alimentary canal, by administering, by the mouth, substances which kill or paralyze the worm, without producing serious symptoms of poisoning in the host. The conditions, however, are so different from those which govern the treatment of infections by parasites of the blood and tissues, and the action involved is so obviously and beyond question a direct poisoning of the parasite, that the inclusion of this type of treatment in this review would be misleading rather than illuminating. The case is different with infections by worms of the blood and tissues: here the problems of treatment closely resemble those encountered in dealing with protistal infections. Such success as has been attained has resulted from the empirical trial of remedies which have been found effective in infections with protozoa or spirochaets. Infection with the guinea-worm can, apparently, be cured by intravenous administration of salvarsan or tartar emetic. (Jeanselme (1919), Montpellier and Ardoin (1919), Macfie (1920).) But the most striking and best authenticated success has been attained in treating

infections with *Bilharzia* (*Schistosoma*) by tartar emetic, injected intravenously. This was tried by Christopherson (1918), (1919), (1920) in 1918, who was induced to make the attempt by his observation of its success in Leishmaniasis. Before this successful trial, a chemotherapeutic attack on *Bilharzia* infections had been regarded as an almost hopeless enterprise, since the symptoms constituting the essential disease are produced, not by the adult worms in the circulation, but by the innumerable, irritating and highly resistant ova, finding their way to the exterior through the tissues of the bladder and the rectum. It is still a matter for surprise that the eggs should be killed by the antimony treatment, and that, with their death, the irritating action should cease. The evidence as to the mode of action is still incomplete. Christopherson describes experiments, which he regards as proving a direct lethal action of the tartar emetic on the ova and the free-swimming embryos (miracidia). It cannot be said that, having regard to the concentrations employed, they carry a strong conviction of the correctness of his view.

This theory of directly lethal action is rendered more difficult by the fact that a number of observers (Diamantis (1917), Mayer (1918), Bonne (1919), Debbas (1920), Erian (1919), Tsykalas (1921)) have made very similar claims for emetine, administered hypodermically or intravenously, as a cure for bilharziasis of the bladder or the rectum. On the other hand, it is at least extremely doubtful (Low and Gregg, Macfie) whether tartar emetic has, on *Filaria* infections, the curative effect which Rogers had suggested.

#### CONCLUSION

In the foregoing pages an attempt has been made at an impartial summary of the facts which must be covered by any general theory of the mode of action of specific chemical remedies for infections. Already in the early days of this type of investigation, when attention was focussed on the effects of certain chemicals on infection with trypanosomes, facts were constantly presenting themselves which could only with difficulty, and with aid of subsidiary hypotheses, be reconciled with the idea that the curative substance acted in virtue of its differential affinity for the parasitic protoplasm. The contrasts between effects *in vitro* and those *in vivo*; the phenomena of acquired resistance, specific for the infected host as well as for the infecting strain of parasites, and showing, at the same time, discrimination between nearly related remedies and community as regards others having no chemical

similarity; these phenomena were, and still are, difficult to bring within the scope of any simple type of explanation. The difficulty has greatly increased during recent years, as the methods of chemotherapy have spread to the treatment of other types of infection. When we are dealing with organisms of one genus, or of a few closely allied genera, the postulation of different chemoreceptors, corresponding to the different kinds of compound found therapeutically effective, though it does not convey more real information than the record of the results observed, does not involve any apparent absurdity. When, however, we try to apply the terminology to the more recent extensions of chemotherapy, and find it necessary to suppose, for example, that the *Bilharzia* worm has antimony-receptors in common with trypanosomes or *Leishmania*, and emetine-receptors in common with the dysentery ameba, it becomes increasingly difficult to use the nomenclature with complete solemnity. Ehrlich's theory will always deserve the credit of having provided a vigorous stimulus to the investigation of problems which, without some kind of working hypothesis, might well have seemed beyond the reach of an experimental attack. That being admitted, it is necessary, on the other hand, to admit that few of the successful results hitherto obtained have been obtained by a consistent application of the theory. Some of them seem, indeed, to be the result of experiments which a serious acceptance of the theory would have discouraged. As new successful applications have become more frequent, their basis has become increasingly empirical. It is difficult to resist the conclusion that a new theoretical foundation is required for further orderly building, and that this will have to take fuller account of the great complexity of the therapeutic process, and especially of the coöperation therein of the infected host. And, if this should mean some measure of reunion between "chemotherapy" and the parent pharmacology, from whose rather unenterprising tradition it claimed to be free, the result can only be to the advantage of therapeutic science.

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## INTERMEDIARY METABOLISM OF CARBOHYDRATES

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Much the greater part of the energy made use of by man and other animals to warm their bodies and to do muscular work is obtained by the oxidation of glucose and other simple sugars, derived from the digestion of various carbohydrates contained in the food. In ordinary diets of human beings from one-half to two-thirds or even more of the oxidizable foodstuffs are sugars and starches, which after digestion are absorbed from the intestine chiefly in the form of glucose. Fructose and galactose are also absorbed and utilized, though in much smaller quantities.

Besides the food carbohydrate, about 60 per cent of the protein amino acids and about 10 per cent of fat (glycerol) (129) are believed to be converted into glucose in the normal course of their metabolism. Fatty acids also, according to the view of some investigators, are convertible into glucose (74), though the burden of evidence appears to be contrary to this conclusion. The metabolism of glucose thus represents, from a quantitative standpoint, much the most important chemical reaction taking place in the body, and for the purposes of this brief survey, its transformations will be regarded as representing the intermediary metabolism of carbohydrate.

In addition to starch and common sugars which yield on hydrolysis or digestion the three hexoses named above, plant tissues used for food by herbivorous animals contain large amounts of polysaccharides which on hydrolysis by acid yield sugars of five carbon atoms, the pentoses. These pentosans undoubtedly furnish a very important source of energy to grazing animals, but it is probable that their digestion is largely in the nature of fermentation by flora of the intestinal tract, with the formation of acids and other products rather than the constituent pentose sugars. Insofar as this is the case the carbohydrate characteristics are lost before the products enter the blood stream. In these animals glucose, glycogen and lactose may be formed by synthesis from the fermentation-digestion products of pentosans, which thus by a

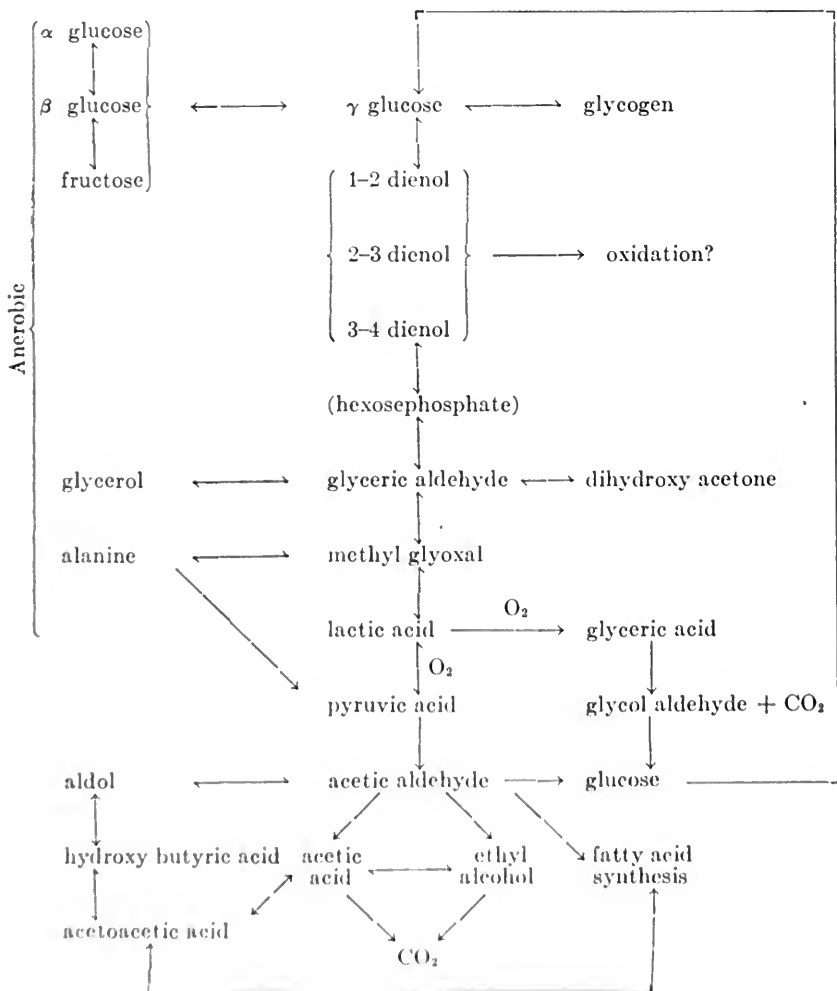
round-about transformation play an important rôle in the carbohydrate metabolism. Pentosans are scarcely if at all digested or absorbed by man, and therefore have little part in human metabolism. When the pure pentoses are fed a part has been found to be excreted unchanged; neither arabinose or xylose are converted into glucose in the body (13). It appears that these sugars and the methyl pentose, rhamnose may be metabolized in small amounts (24), (122), though nothing is known of the stages of their oxidation.

Glucose, whether derived from ingested sugars and starch or formed from amino-acids or other substances in metabolism, may be burned to carbon dioxide and water, with or without an intermediate conversion into glycogen, or may be converted into fat. The chemical reactions by which these transformations are accomplished have long been subjects of investigation, and many interesting facts are known, but the fundamental problems remain unsolved. The trend of opinion at present is toward the view that the general course of the reactions in animal cells is very similar to, and many of the intermediates the same as, the fermentation of sugar by yeasts. From this point of view the subject was reviewed by Neuberg in 1913 (151), (152) and later work by Meyerhof, Embden and others has been interpreted as confirming this similarity. The present discussion will however be limited to the reactions of glucose in the animal body.

The path of glucose metabolism does not lie in its direct oxidation, or in its direct synthesis to fatty acid, but the molecule first undergoes progressive conversion into other much more reactive substances and it is these products resulting from molecular rearrangements which are finally oxidized with the liberation of energy, or are synthesized to fat and other substances. The first step appears to be the transformation of ordinary glucose into a more reactive isomeric form, which may undergo condensation to polysaccharides (glycogen) or with phosphate to form salts of a hexose phosphoric acid. These derivatives of the original glucose, or their nascent hydrolytic products, "dissociate" into three carbon fragments which are highly reactive and correspondingly unstable, and under certain circumstances appear in the body as lactic acid. According to the now prevalent view lactic acid represents the main intermediate in glucose metabolism and it is with this substance that oxidation actually begins. Although the belief that glucose *oxidation* proceeds through lactic acid may be open to question, there is conclusive proof that the conversion,  $\text{glucose} \rightleftharpoons \text{lactic acid}$ , does normally take place in both directions in the body, and that the reactions

concerned in this transformation are fundamentally related to carbohydrate metabolism.

As a basis for the following discussion the reader is asked to consult the following diagram. Some of the reactions indicated are more or less hypothetical and the diagram is intended only as an aid in orienting ourselves in the maze of evidence which requires consideration.



It will be noted that the first group of reactions at the top of the diagram involve only reversible rearrangements within the glucose molecule and its condensation to polysaccharides (glycogen), in which little

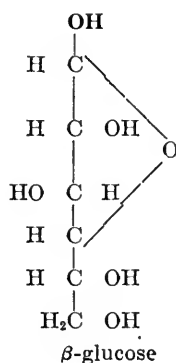
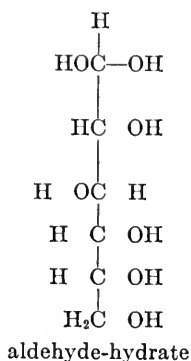
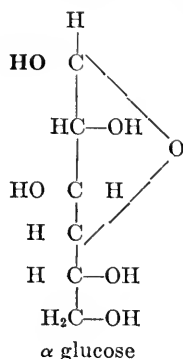
or no energy change takes place. Recent evidence indicates that these transformations although not extensive as to molecular structure may nevertheless be of fundamental importance.

The reactions of the second group from glycogen or  $\gamma$  glucose to lactic acid are probably also reversible and involve no oxidation and but little energy change, but do involve a splitting of the six carbon molecules into three carbon fragments. The loss of energy in the sum of the reactions from glucose to lactic acid (including neutralization with bicarbonate or phosphate) is at most about 190 calories per gram of lactic acid (141) which is only about one-twentieth of the calorie value of the glucose from which it was derived.

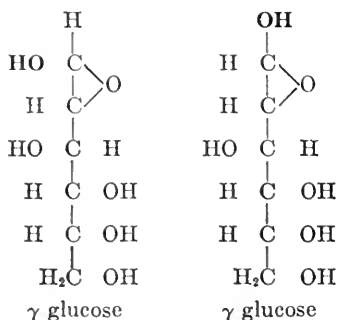
The third group of reactions concerns the oxidation of the three carbon dissociation fragments of glucose, and it is in these stages that the bulk of the energy is liberated. Or it may be that the oxidation is not of lactic acid and its products, but of an earlier derivative of the parent glucose. The oxidative reactions are, of course, exothermic and are, in the main, irreversible in the body.

We may first consider the evidence as to different forms of glucose.

**THE DIFFERENT FORMS OF GLUCOSE.** It has long been known that the optical activity of freshly prepared solutions of ordinary glucose gradually becomes less, and only after some hours attains a value corresponding to the specific rotation of  $+52.5^\circ$ . This phenomenon (mutarotation) which is exhibited also by other reducing sugars, studied especially by Lowry (127) and by Hudson (96), is due to the slow conversion of  $\alpha$ -glucose, a form predominating in ordinary solid preparations of the sugar, into  $\beta$ -glucose. The final rotation is the result of an equilibrated mixture of the two forms, which were isolated by Tanret (190). The formulae generally assigned to these forms are given below and represent butylene oxides corresponding to the  $\alpha$ - and  $\beta$ -methyl glucosides prepared by Fischer.



The conversion of one form into the other is believed to take place through the intermediate formation of the aldehyde structure by the breaking of the butylene ring and its reformation. Although slow at low temperatures and in neutral solutions, the conversion is rapid on warming and is practically instantaneous in the presence of even very slight alkalinity.



Besides these ordinary forms there is good evidence that glucose exists also in much more reactive modifications. Fischer (59) and Irvine (98) have identified two additional methyl glucosides as the derivatives of an ethylene oxide structure of glucose, designated  $\gamma$  glucose. The parent glucose according to Irvine, Fyffe and Hogg (98), (99), and Armstrong and Hilditch (5), (6), readily undergoes condensation, is rapidly oxidized by permanganate, and is much more reactive than ordinary glucose. Although  $\gamma$  glucose has not been isolated, its existence and its greater reactivity appear to be established. These more reactive forms have acquired very great biochemical significance through several recent discoveries which suggest that the  $\alpha$  and  $\beta$  butylene-oxide forms must be converted into the  $\gamma$  forms before glucose is utilized in the body. Hewitt and Pryde (85) have found that when solutions of ordinary glucose are injected into a loop of intestine it is rapidly converted into a form having lower optical activity and greater reactivity than the equilibrated mixture of the  $\alpha$  and  $\beta$  forms. On withdrawal from the intestine, the optical activity slowly rises until it corresponds with the rotation of ordinary glucose. They conclude that the intestinal mucosa accomplishes the transformation of  $\alpha$  and  $\beta$  into  $\gamma$  glucose.

These important observations have been very recently extended by Winter and Smith (195) who find that the sugar of the blood of normal animals and men, when examined as promptly as possible after its separation at low temperature from protein and salts, has an optical

activity much below that which corresponds to ordinary equilibrated glucose. On standing the optical value rises until after a day or two it becomes constant and agrees with the value expected from the determination of glucose by copper reduction methods. They were unable to detect fructose in the separated blood sugar. The phenyl hydrazine osazone had the melting point and the crystal form of glucosazone.

Furthermore, blood sugar during alimentary hyperglycemia following the ingestion of 150 grams of fructose and after 100 grams of glucose, showed the same discrepancy between optical rotation and copper reduction values; the copper values remained constant, while the optical values increased until the two agreed. Presumably, therefore, both glucose and fructose are transformed during absorption or soon thereafter into the same form. The sugar obtained from the blood of human diabetics on the other hand did *not* show the low optical rotation, but agreed with the copper reduction values. In one case the initial rotation of the diabetic sugar was *higher* and gradually decreased to the copper value. But after the injection of "insulin" (9), causing a decrease of hyperglycemia and of glycosuria, the sugar extracted from diabetic blood was found to be *similar to that of normal blood*, the initial rotation being lower than corresponds with the copper reduction, and increasing on standing (34). The authors interpreted their observations as indicating the conversion in the body of the normal subject of  $\alpha$ - and  $\beta$ -glucose into the more reactive  $\gamma$ -form having a lower specific rotation,—a conversion which is not accomplished by the diabetic organism. The same workers have expressed the hypothesis that this conversion is essential for the oxidation of glucose, and that the inability to effect this transformation is the basic defect in diabetes. They report that preparations of pancreas ("insulin"), when added to liver extracts bring about a decrease in the optical activity of glucose which they interpret as being due to its conversion into the  $\gamma$ -form (196).

The observations recall the important results of Admont Clark (17) who found that on perfusion of dog's pancreas with Locke's solution containing glucose, the optical activity of the solution is reduced but its copper reducing power is unaltered. After acid hydrolysis, or standing with antiseptics, the loss of optical activity was regained. Perfusion of heart, spleen or kidneys did not cause this loss of optical power. When however the beating heart was perfused with Locke's solution which was passed also through the pancreas, there was a decrease in reducing power as well as of optical activity, both being in part

regained on acid hydrolysis. Besides a consumption of glucose, a part was converted into a substance having lower optical activity than ordinary glucose. The osazones prepared from pancreas perfusates had melting points distinctly different from that of glucosazone, being from 200.5°C. to 203°C., while after acid hydrolysis of the same perfusates, the osazones then obtained melted from 203.5° to 206.5°. Clarke concluded that the pancreas furnished to the perfusing fluid an enzyme which changes glucose into a "simple form of polysaccharide" with lower optical activity but of the same reducing power as glucose. The heart was able to utilize some of the sugar after it had been transformed by the pancreas enzyme, and a part was further condensed to polysaccharides, the optical activity and reducing power of which were increased by acid hydrolysis.

In addition to demonstrating a reversible decrease in the optical activity of glucose on perfusion through pancreas, which, in the view of Hewitt and Pryde and of Winter and Smith, may be explained by its transformation into  $\gamma$ -glucose, Clark's results also point clearly to the necessity of this transformation before glucose can be oxidized by the heart muscle. Recent experiments by Hepburn and Latchford (84) demonstrate an increased rate of glucose consumption by the beating heart when insulin is added to the perfusion fluid.

Referring to our diagram it will be noted that the investigations cited above have to do especially with the transformation of  $\alpha$ - and  $\beta$ -glucose into another form, possibly  $\gamma$ -glucose. According to Irvine, one of the characteristics of  $\gamma$ -glucose is its tendency to undergo condensation. This suggests that it may be the  $\gamma$ -glucose, formed by the intervention of pancreas activator, which forms glycogen. Banting, Best, Collip, Macleod and Noble (9) find that the amount of liver glycogen in depancreatized dogs is very markedly increased by insulin injections, whereas, as is well known, the amount of glycogen in diabetic livers is ordinarily small. Macleod (132) takes this to mean that glycogen is an essential preliminary stage in sugar utilization, and that glycogen is not formed in the absence of the pancreatic hormone. This is in line with the results of Barrensechen (10), who found that on perfusion of the livers of depancreatized dogs, added glucose and fructose do not form glycogen, while this formation does take place on similar perfusion of livers of normal animals.

From the evidence cited above the hypothesis appears attractive, that  $\alpha$ - and  $\beta$ -glucose is converted under the joint influence of tissues and of the pancreas hormone into  $\gamma$ -glucose, which may then be poly-

merized to glycogen or transformed into other derivatives capable of oxidation, both of which fates are closed to the molecular configuration of ordinary glucose. According to this view the effect of the pancreas hormone is located with the early molecular rearrangements within the glucose molecule, perhaps in initiating its enolization, and especially the formation of 1-2 dienol, which may be regarded as similar to  $\gamma$ -glucose.

Brief mention may be made of another sort of condensation which may or may not have to do with glycogen formation. It will be recalled that Hirsch (91), Arnheim and Rosenbaum (7) and especially Cohnheim (23) observed a disappearance of glucose by the joint action of muscle juice and pancreas extracts. The observation was confirmed by Hall (80), Dewitt (35) and Levene and Meyer (114). The last named investigators, however, showed that the loss of copper-reducing power was the result not of "glycolysis" but of a condensation. After the action of muscle + pancreas extract upon strong glucose solutions, they were able to isolate an osazone, which on analysis appeared to be that of a disaccharide. After the digestion had taken place with a lowering of reducing power, hydrolysis of the resulting sugar solution by acid, or hydrolysis by the tissue enzymes after dilution, increased the copper reduction values to that of the controls before digestion. Similar condensation was observed of fructose, but not with mannose, xylose, ribose and lactose. The condensation of glucose was not found to take place by muscle or other tissues alone, but required the addition of pancreas extract (or, in case of the dog, spleen extract, (117)).

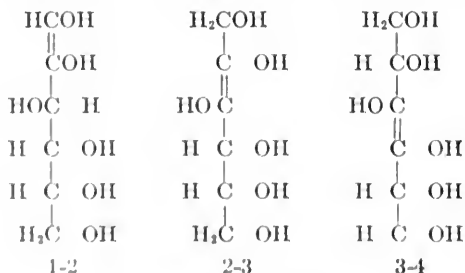
This phenomenon appears not to have been further investigated, and its significance is at present not clear. The effect of Levene and Meyer's (117) results has been to cast doubt upon its relation to glucose catabolism, but in view of the participation of pancreas extracts in accomplishing the observed condensation, and in the light of the more recent work suggesting that the action of the pancreas hormone may be upon the *preliminary stages* of glucose transformation, it seems possible that it may, after all, be related to a phase of glucose metabolism. It should be noted, however, that the conditions under which this type of condensation has been observed are very different from those under which either glycogen formation or "glycolysis" by conversion to lactic acid have since been found to take place. (Antiseptics, long incubation and high sugar concentration.) Sterile suspensions of leucocytes or of kidney tissue in phosphate solutions convert added glucose, fructose, or mannose into d-lactic acid (115, 116, 117), but the presence of toluol wholly prevents this action.

*The action of alkalis on glucose.* Although relatively stable in acid or neutral solutions, under which circumstances glucose requires strong reagents for its oxidation, it becomes at once highly reactive in the presence of alkalis and will absorb atmospheric oxygen (135). Even if oxygen be excluded the optical activity declines (142) and the sugar is converted into a large number of different substances. The reactions which take place in alkaline solutions have been found to be of great complexity. Some of the points resulting from these extensive investigations may be stated as follows.

In exceedingly dilute alkali there occurs a very rapid transformation of freshly dissolved glucose or other sugars, with a change in optical rotation of the solution, the change being due merely to the partial conversion of  $\alpha$ -glucose into the isomeric  $\beta$ -form (mutarotation)

In somewhat stronger alkali the initial sugar is gradually converted into a mixture of all of the members of that series (Lobry de Bruyn phenomenon). d-Glucose, for example, gives a mixture of six isomeric hexoses including d-glucose, d-mannose and d-fructose, which result, according to Nef, from the formation of 1-2, and 2-3 d-glucose dienols, the H and OH groups on the second and third carbon atoms being thereby rendered mobile. In moderately dilute alkali the carbon chain is however not split (147).

With stronger alkali, however, there takes place, besides the rearrangements above mentioned, very extensive disruption of the molecule and the formation of more than a hundred different substances (147). The hexoses split into *a*, formaldehyde and aldopentose (123); *b*, dioses and aldo tetroses; and *c*, two trioses, by the intermediate formation of 1-2, 2-3 and 3-4 hexose dienols, and their spontaneous decomposition.



d - glucose di-enols

Nef and his pupils (75, 147, 187, 192) isolated from alkaline sugar solutions products of each type of decomposition, though there is reason to believe that the split into three carbon fragments is quantitatively

the predominant reaction, and that the single carbon methylene dissociation is very slight (unless oxidation occurs). The products formed by the splitting of the dienols are very unstable and reactive substances. In the absence of oxygen they form salts and polymerize to sugar resins or undergo an internal Cannizzaro rearrangement with the irreversible formation of "saccharinic acids," of which lactic acid is quantitatively the most important (147 d). *But in the presence of abundant oxygen the "dissociation products" are oxidized and neither resins nor saccharinic acids (lactic) are formed.*

This behavior of glucose in alkaline solution must be of the greatest importance to an understanding of the course of its breakdown in living cells, since there are very striking similarities in the reactions under the two conditions (200). In the test tube glucose is quite inert toward oxygen until its molecule is opened up by the action of alkali and converted into other very unstable and easily oxidizable substances; and in the animal body it is likewise altogether resistant to oxidation unless a combination of agencies, among which is the action of a pancreas hormone, converts it into reactive fragments whose identity remains unknown. Whatever the reactive derivatives may prove to be, whether formed by alkali in the test tube, or by biochemical agencies in the cells of the body, they are in both cases converted into the same substance, lactic acid, unless they are first oxidized. Lactic acid is thus the chief product of the anerobic catabolism of glucose, alike in cells and in vitro, and a knowledge of the unstable derivatives of glucose which are precursors of lactic acid is fundamental to an understanding of glucose metabolism.

The formation of lactic and other acids from sugars by the action of strong alkali, discovered by Hoppe-Seyler (94) has been studied by many workers (Kiliani (104), Buchner and Meisenheimer (15), Meisenheimer (140), Nef (147), Framm (65), Upson (192), M. Oppenheimer (159) and the quantitative yield has been reinvestigated recently in the writer's laboratory by T. E. Friedemann (66).

When warmed or allowed to stand in an excess of 0.2 to 1. N sodium or potassium hydroxide each molecule of glucose forms a total of about 1.6 molecules of (mono-basic) acids of which about one-half is lactic acid, very small amounts of  $\text{CO}_2$  and formic, and the rest, non volatile ether-insoluble "saccharinic acids" (140, 158, 66). The maximum yield of lactic acid corresponds to slightly less than one molecule from each molecule of glucose, which suggests that the main dissociation is into

two 3-carbon fragments, only one of which in the test tube is converted into lactic acid.<sup>1</sup>

The main initial products are probably glyceric aldehyde or di-hydroxy acetone, formed by splitting of a 3-4 di-enol, half of which is transformed by loss and addition of water into methyl glyoxal, which is known to be immediately and completely converted by alkali into lactic acid (33). This interpretation of the course of the reaction is based upon the facts, 1, that glyceric aldehyde and di-hydroxy acetone give, when acted upon by alkali, the same amount of total acids

TABLE 1

*The action of alkali on glucose, glyceric aldehyde and glycol aldehyde: alkali "dissociation", with subsequent and simultaneous oxidation by hydrogen peroxide (T. E. Friedemann)*

TREATMENT	SUGAR	TOTAL ACID	LACTIC ACID	FORMIC ACID	OXALIC ACID (AFTER OXIDATION BY ALKALINE $\text{KMnO}_4$ )
		cc. N	mols.	mols.	
"Dissociation" by alkali	1 mol glucose	1.6	0.8	0.09	1.8
	2 mols glyceric aldehyde	1.5	0.8		1.8
	3 mols glycol aldehyde	1.45	0.6		1.8
"Dissociation" followed by oxidation by $\text{H}_2\text{O}_2$ .	1 mol glucose	2.2	0.8	0.5	1.6
	2 mols glyceric aldehyde	2.55			
	3 mols glycollic aldehyde	2.1			
Simultaneous "dissociation" and oxidation	1 mols glucose	5.0	0	4.0	0.8
	2 mols glyceric aldehyde	5.8	0	5.6	0.09
	3 mols glycol aldehyde	5.7	0	5.4	
Untreated	1 mol glucose				3.0

and of lactic acid, as does glucose (158, 66) and 2, that methyl glyoxal may be detected among the products formed from glucose by the action of alkali. Pinkus (167), Nef (147), Neuberg and Oertel,

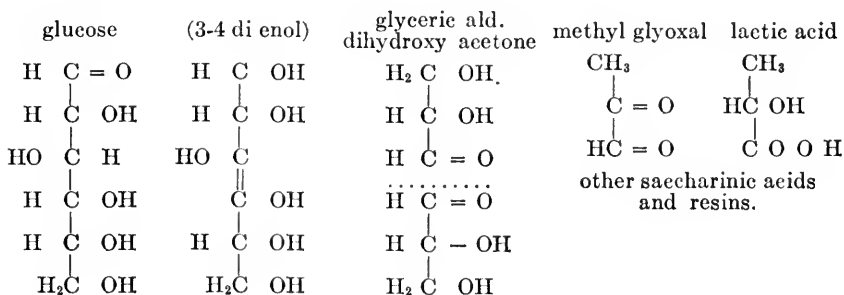
<sup>1</sup> The same inference can be drawn from the amount of oxalic acid formed by oxidation with alkaline permanganate. When oxidized before "dissociation" the 6-carbon chain of glucose yields 3 molecules of oxalic acid, while if oxidized by  $\text{KMnO}_4$  after dissociation by alkali, slightly less than 2 molecules of oxalic are obtained, one from each 3 carbon fragment (see tables).

Although with alkali the maximum of lactic acid corresponds to only one half the carbon of glucose, the conversion in the animal body is believed to be complete.

Windaus and Knoop (194), Dakin and Dudley (26), Fernbach and Schoen (58).

In the accompanying table are given some results obtained by T. E. Friedemann which show that the amounts of total acids and of lactic acid from the alkali dissociation of glyceric aldehyde, and indeed of glycol aldehyde,<sup>2</sup> are almost identical with those of glucose.

It appears, therefore, that in half normal alkali and in the absence of oxygen, the dominant decomposition of glucose is:



Wohl (199) has suggested a slightly different course with the formation of methyl glyoxal glyceric aldehyde aldol, which then splits to 3-carbon fragments.

The *oxidation* of glucose in alkaline solution takes a different course from that outlined above. It has already been noted that the oxidation is *not* by way of lactic acid, but takes place *before* lactic acid is formed. If directly oxidized by hydrogen peroxide in alkaline solution, glucose yields no lactic acid, but 4 molecules of formic acid and 1 of glycollic acid (66); but if first dissociated by alkali the lactic acid formed is not oxidized by subsequent treatment with alkali (see table 1). Which of the precursors of lactic acid is the starting point of glucose oxidation in alkaline solutions? The following facts appear to indicate that it is not methyl glyoxal but a still earlier derivative.

<sup>2</sup> The fact that 3 molecules of the two-carbon glycol aldehyde when treated with alkali form nearly as much lactic acid as one molecule of glucose or two of glyceric aldehyde is additional evidence of the polymerization of sugars long known to take place in alkaline solution (16), (126), (59).

Evidently the glycol aldehyde is in large part condensed to tetrose or hexose which then dissociates into three-carbon fragments; and this type of reaction illustrates the processes involved in the formation of glucose (and lactic acid) from the derivative of glycine in the body.

The conversion of methyl glyoxal to lactic acid in alkaline solution is almost instantaneous, far more rapid than the rate of lactic acid production from glucose. The rate of lactic acid production is, therefore, not limited by the rate of formation of methyl glyoxal but by earlier reactions. But the rate of glucose decomposition is markedly increased by oxygen (65). Judged by total acid production, loss of optical activity, or loss of reducing power, the reaction is practically complete in 0.5N KOH and  $\text{H}_2\text{O}_2$  at  $37^\circ\text{C}$ . in 4 hours, while the same relative stage in the reaction without peroxide is reached only after 16 hours (66). The increase in rate caused by oxygen must be due to the oxidation of an earlier derivative and one formed *much more rapidly* than methyl glyoxal.

There is evidence also to suggest that the oxidation begins at a point even before glyceric aldehyde. Friedemann's results (see table 1) show that while the end-products of *dissociation* by alkali are substantially the same for glucose and for glyceric aldehyde, the products of *simultaneous oxidation and dissociation* are different, in that glyceric aldehyde gives more formic acid and little or no glycollic acid (oxalic) compared with 0.8 molecule of glycollic (oxalic) acid from glucose. This difference suggests that in spite of the fact that *alkali dissociation* gives the same products, the starting points of *oxidation* are different. This can only mean that in the main, the glucose derivative which is oxidized is *before* the stage of glyceric aldehyde.

That glucose itself is not directly oxidized is shown by an initial lag in the reaction, during which period some transformation is doubtless taking place; furthermore, by direct oxidation of glucose, the first product would be gluconic acid, which is not formed. We are thus left with the notion that oxidation begins with some intermediate between glucose and glyceric aldehyde. There appears to be no clear evidence as to what this intermediate may be, though we may recall the conceptions of Nef (147) and of W. Löb (123) that single carbon fragments are progressively split off from 1-2 di-enols (Nef, "Hydroxy methylene dissociation") and it may be imagined that the removal of these fragments by oxidation diverts the reaction from the otherwise predominating formation of 3-4 di-enols and their splitting to trioses. Or it may be supposed that the 1-2 dienols ( $\gamma$ -glucose?) are oxidized through the stage of the corresponding osones.

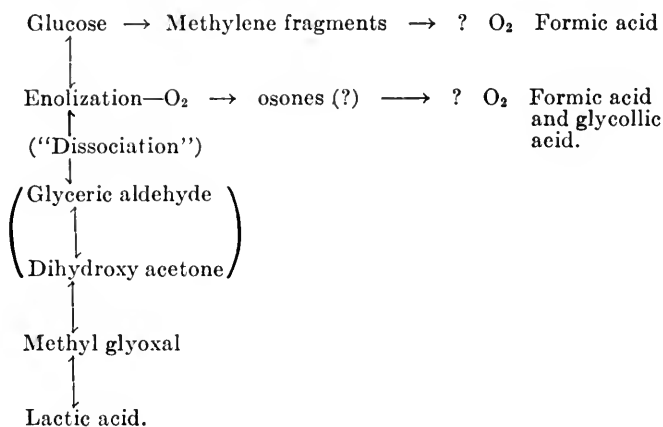
According to W. Löb and Beysel (123), and Witzemann (198), phosphates accelerate the oxidation by  $\text{H}_2\text{O}_2$  even at very low alkalinity (at which reaction lactic acid formation does not occur). Experiments

by Harden and Henley (81) lead them to question whether the action is specific, but in view of the possible similarity of this *in vitro* effect of phosphates to their specific effect in yeast fermentation and in the formation of lactic acid in muscle, the subject merits more thorough investigation.

Although the exact identity of the first oxidizable derivative of glucose must be left undecided, there appears to be no escape from the conclusion that in alkaline solution oxidation begins at the early stages of dissociation and before the molecule is split into 3-carbon fragments. If this view is correct, it is of interest in connection with the question whether the main path of oxidation in the body has its beginning with a derivative *before* or *after* the formation of lactic acid and its 3-carbon precursors.

The reactions from glucose to lactic acid may be regarded as reversible. Although in alkaline solution lactic acid is not converted to methyl glyoxal, Dakin (26) shows that this does occur in acid solutions. When allowed to stand with p-nitrophenyl hydrazine, the osazone of methyl glyoxal is precipitated. The *in vitro* synthesis of hexose from glycerose is established by the well known work of Fischer. The conversion of methyl glyoxal to triose has not been observed *in vitro*, but the transformation of methyl glyoxal (26) as well as of lactic acid (26), (134) and glyceric aldehyde (177), to glucose is known to take place in the body.

The predominating reactions in the decomposition of glucose and its oxidation in alkaline solution may be represented in outline as follows. Besides the steps indicated, there are other dissociations and polymerizations, details of which are less well known.



THE REVERSIBLE CONVERSION,  $\text{GLUCOSE} \longleftrightarrow \text{LACTIC ACID}$  IN THE BODY. The belief that lactic acid, (the presence of which in muscle was discovered by Berzelius), has its origin in carbohydrate was expressed by Liebig in 1847 (121), who formulated the view that sugar is converted in the blood into lactic acid which is oxidized as fast as formed, except where oxygen is lacking. Ever since that time lactic acid has been associated with muscular contraction, and the work of Du Bois Reymond, of Nasse and others clearly pointed to glycogen as its source. The evidence was reviewed by Nasse in 1879 (146) with the conclusion that the source of the lactic acid in muscle is, directly or indirectly, glycogen, which during contraction or in rigor is converted by a "spaltungsprozess" and not by oxidation, into sugar and lactic acid with the evolution of heat and work.

Although the methods used in the earlier investigations are, according to present standards, open to question and the reliability of the analytical results scarcely justified the conclusions, it is of interest to note that after a period of doubt as to the origin of lactic acid, the most modern view has returned to the early conception. There is no longer question that lactic acid of muscle is formed from glucose or glycogen, though the search continues for the unstable intermediate. Hermann's "inogen" has been abandoned, only to be replaced by Embden's "lactacidogen."

The close relation of lactic acid to carbohydrates was early indicated by its formation from sugar by fermentation (the inactive acid), and was greatly strengthened by the discovery of Hoppe-Seyler (94) already referred to that large amounts of this acid are formed by the action of strong alkali on glucose and many other sugars. Hoppe-Seyler realized that the formation of lactic acid by the action of alkali occurs in the absence of oxidation, a point of view which led to later work in his laboratory by Araki (3), (4) and Zillesen (202) who showed that the excretion of lactic acid is the result of asphyxia. Araki found lactic acid and sugar in the urine of animals when asphyxiated or poisoned with substances ( $\text{CO}$ , morphine,  $\text{HCN}$ , strychnine, phosphorus, arsenic) which were believed to produce tissue asphyxia and concluded that the origin of the lactic acid was glycogen.

The formation of lactic acid from glucose in the whole body was also indicated by Mandel and Lusk (134) who found the acid excreted by dogs poisoned with phosphorus to disappear from the urine after administration of phlorhizin and resulting glycosuria. Later Von Fürth (71) showed that feeding glucose to phosphorus poisoned rabbits in-

creased the lactic acid excretion, while the previous removal of glycogen reserves prevented a lactic acid excretion which otherwise resulted on exposure to cold (72). Similarly Sass (179) found that strychnine convulsions in depancreatized dogs produces less change in blood alkalinity (due to less formation of lactic acid) than in normal dogs.

The reverse transformation, lactic acid  $\rightarrow$  glucose was demonstrated by Lusk and Mandel (134) and by Embden and Salomon (57) to occur when lactic acid is fed to phlorhizinized and depancreatized dogs. Under these conditions the conversion is believed to be quantitative, two molecules of lactic acid giving one of glucose. It may therefore be concluded that the animal body readily accomplishes the conversion glucose  $\longleftrightarrow$  lactic acid, in both directions; and it has been shown that the same reactions occur in isolated surviving tissues, muscle, liver, kidney and blood.

*Muscle.* Representing not only the largest single fraction of body substance, but the tissue for the performance of mechanical work, the greater part of carbohydrate metabolism occurs in muscle; and lactic acid is the agent through which the energy of glucose is transformed into heat and the work done by muscular contraction. The notable recent developments in knowledge of the mechanism of muscular contraction and of its energy conversion have been ably reviewed by A. V. Hill (87) in these REVIEWS and we shall mention only briefly the more important work bearing upon the source and fate of lactic acid.

Fletcher and Hopkins in 1907 (63) showed that in the freshly excised resting frog muscle the amount of lactic acid is small, but gradually increases, under *anaerobic* conditions, as long as irritability remains. When exposed to oxygen lactic acid is not formed. As a result of stimulation, heating, mechanical or chemical injury, the rate of lactic acid production is enormously increased. After accumulating lactic acid in an atmosphere of hydrogen a large part of the acid disappears on exposure to oxygen. This "oxidative removal" of lactic acid did not take place after hashing or other injury of the muscle, and the authors concluded that the oxidation process is not the result of simple chemical reaction but is dependent upon the events of the normal life of the muscle. The source of the lactic acid and its fate on oxidative removal were not considered by Fletcher and Hopkins, except to suggest that the amount formed is "quite out of proportion to its glycogen content."

From an analysis of the time relations of heat liberation during and following muscle contraction, Hill (83), (87) showed that the *initial* rapid heat production is the same under anaerobic and aerobic conditions

thus indicating that oxidation is not concerned with the act of contraction during which lactic acid is formed. In the absence of oxygen the lactic acid persists and there is no further (or a small) heat production; but in the *presence* of oxygen, the lactic acid disappears during the recovery phase following contraction, with a simultaneous liberation of heat. *The formation of lactic acid in muscle is therefore a very rapid process taking place in the absence of oxygen and without the liberation of much heat, and it is during the removal of the lactic acid that oxygen is consumed, heat liberated, and the greater part of the oxidation occurs.* It seemed probable that the lactic acid was oxidized (163), though Hill found the total heat evolved was sufficient to account for only about one-fourth the lactic acid which disappeared and its fate was therefore doubtful.

In 1911 Fletcher (62) reinvestigated the formation of lactic acid during autolysis of hashed muscle preserved with chloroform water and toluol, under which conditions a number of workers (143), (103), (174), (97) had observed a continued production of lactic acid to occur. Fletcher found with mammalian muscle a rapid production during the process of hashing and that little or no further increase occurred on autolysis with antiseptics unless the preparations became contaminated with bacteria. He was unable to obtain any increase of lactic acid on the addition of glucose or glycogen and concluded that the results of Ransom (168) as well as of Stocklasa (188) who claimed a fermentation of glucose by muscle and other tissues, with the formation of lactic acid,  $\text{CO}_2$  and alcohol, were erroneous and probably due to bacterial action. According to Fletcher, there was no "glycolytic" formation of lactic acid in muscle, it being probably derived from some other "specific unstable precursory material" the amount of which is determined by "previous intra-cellular events."

A similar opinion was expressed by Embden, Kalberlah and Engel (49) who found that muscle press juice, prepared from frozen dog muscle, (and containing at the start almost a maximum of lactic acid) showed a further slight but distinct increase in lactic acid on incubation for one hour. The maximum increase of lactic acid was attained within 30 minutes, was favored by the addition of sodium bicarbonate, and was wholly inhibited by acid (105). Since added glucose or glycogen were not converted to lactic acid Embden and collaborators believed that their results excluded direct relationship between lactic acid and carbohydrate, and that the precursor must be some other unknown substance which they termed "lactacidogen."

Brief mention may be made at this point of the interesting investigations which have grown out of the discovery of "lactacidogen,"—an adequate account would carry us beyond the bounds of this paper. For a review see E. Schmitz (181). Following the observation of Embden, Griesbach and Schmitz (46) that an approximately equimolecular increase of inorganic phosphate accompanies the formation of lactic acid in muscle juice, and that added hexose phosphate led to an increase of lactic acid, it was concluded that "lactacidogen" is a hexose phosphate, and Embden and Laquer (51), (52) isolated from muscle an osazone identical with that of hexose phosphoric acid previously isolated from yeast by Lebedew (113) and Young. Embden, Schmitz and Meineke (55) confirm the parallel between lactic and phosphoric acid formation in dog and rabbit muscle and find that food has little influence on the amount of "lactacidogen." Even fasting and administration of phlorhizin cause no decrease, though a decrease is observed in muscles of phlorhizinized dogs after strychnine convulsions. In accord with their conclusion that "lactacidogen" is the "Tätigkeit-substanz" of striated muscle, an increase of muscular power is claimed to result from the ingestion of sodium phosphate (44) and an increased urinary excretion of phosphate is observed a few hours after exercise (43). The amount of organic (lactacidogen) phosphate has been determined in various muscles of different species (20), (21), (22), (40), (1), (2), (130), (160), (193), in relation to muscular activity. A few reports have even appeared concerning therapeutic results with sodium phosphate, its use being suggested by the work of Embden and his co-workers (cited in (181)).

It appears to be established that phosphoric acid is intimately concerned with lactic acid production in muscle, and with muscular contraction. In the form of "lactacidogen" it is doubtless a component of a precursor of lactic acid, though the first product may be methyl glyoxal. And if the view be correct that glucose oxidation takes place via lactic acid, phosphoric acid is equally concerned with carbohydrate metabolism. This is the view of Neuberg (152), Embden (39), Hopkins (93) and others. The fact, however, that the "lactacidogen" content of muscles, according to Embden, bears no relation to diet and therefore to the rate of glucose combustion, suggests caution in adopting the belief that it is concerned with the *oxidation* of carbohydrate.

Returning to the question of the ultimate origin of lactic acid in muscle, Laquer (111) showed that the "acid maximum" is self limited by its own acidity, and that when suspended in bicarbonate or phosphate

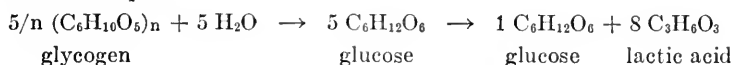
solutions, frog muscle at 30°C. forms lactic acid from added glucose as well as glycogen, while at 45°C. only glycogen and hexose phosphate give rise to lactic acid. After repeated freezing in liquid air the power to convert glucose, fructose, mannose, sorbose and maltose to lactic acid is lost, though a formation of acid from glycogen or hexose phosphate may be retained. The precursor is therefore not glucose or any of the other sugars mentioned, but is some other reactive form, available from glycogen, and into which glucose must be transformed before it can give rise to hexose phosphate and lactic acid (111), (112). According to these results the most sensitive stage of the reactions between glucose and lactic acid—the stage which is first lost—is the conversion of glucose to some active form related to glycogen, rather than the later stages of the reactions. It will be recalled that evidence has been cited which indicates that it is an early stage of glucose transformation which is lacking in diabetes.

In a series of very skilful investigations, Meyerhof (141) has proved that, when suspended in disodium phosphate solution, frog muscle, in an atmosphere of hydrogen, may convert nearly all of its glycogen and lower carbohydrate into lactic acid. He finds close agreement between the amount of total glucose which disappears and of lactic acid formed. The addition to chopped muscle of hexose phosphate, glycogen, glucose and other hexoses does not increase the *rate* of lactic acid formation, but when added at a time when the muscle carbohydrate has been used up, additional lactic acid is formed unless the properties of the living tissues are too far destroyed. The conversion takes place not by an autolytic process but by a continuation of the “*vitalen Stoffwechsels*,” and while this continues glycogen and hexose phosphate are not superior to glucose.

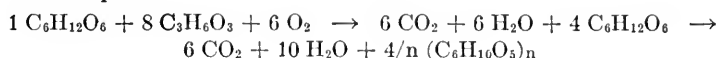
More remarkable than the proof of the origin of lactic acid is the discovery by Meyerhof that the fate of the lactic acid, shown by Fletcher and Hopkins to disappear when the muscle is exposed to oxygen (oxidative removal), is, chiefly, *its conversion to glycogen*. This has been confirmed by Foster and Moyle (64) in Hopkins' laboratory. On repeating the experiments of Parnas who found that the amount of oxygen absorbed during oxidative recovery was sufficient to oxidize all of the lactic acid, Meyerhof (141) showed that the oxygen absorption could account for the oxidation of only one-fourth of the acid which disappeared. Furthermore, he finds, in confirmation of Hill, that the heat evolved during contraction and recovery amounts to about 900 calories for 1 gram of lactic acid formed and removed, an

amount which corresponds with the heat of combustion of  $\frac{1}{4}$  of 1 gram of lactic acid (or of glucose or glycogen). Meyerhof, therefore, concludes that the chemical reaction for the burning of a glucose molecule in muscle may be written as follows.

I. Anoxidative phase



II. Oxidative phase



Four molecules of glucose are converted to 8 molecules of lactic acid and back again to glycogen, with the simultaneous combustion of 1 molecule of glucose, to furnish the energy needed for the transformation. Whether it is actually glucose which is burned or some of the lactic acid, Meyerhof leaves undecided. But his results appear to prove that the fate of the greater part of the lactic acid is *not oxidation*, but conversion to glucose or glycogen; and the idea conveyed by his equation is that *all* of the lactic acid is so converted, the heat being formed by the oxidation of glucose. This is a very fundamental point, for if *all* of the lactic acid formed be reconverted to glucose, *lactic acid would not be a stage in the path of glucose oxidation* in muscle. Meyerhof finds that the ratio of total lactic acid which disappears, to extra heat evolved or oxygen absorbed corresponds to 4 molecules disappearing: 1 molecule oxidized. Hartree and Hill (83) find ratios from 4.9:1 to 6:1. Oxygen is essential to the disappearance of the lactic acid but at most only 1 out of 4 or 6 molecules can be oxidized, the rest reappearing as glycogen or glucose. In spite of oxidation (and only in the presence of oxygen) the *predominant* reaction is the formation of glycogen, which if it occur via triose, *does not involve oxidation*. It appears to be a very paradoxical situation. The course of the reactions is not established and their mechanism is wholly unknown. It is referred to by Embden (47, p. 143), Parnas (163) and Meyerhof as a "coupled reaction" by which the lactic acid is converted to glycogen at the expense of energy derived from the oxidation of a common intermediate—lactic acid or glucose or perhaps of other substance. Hill appears to regard lactic acid as the substance oxidized (83), and with Lupton (88) has recently reported very interesting studies of the relation of lactic acid formation and its oxidation to muscular work and efficiency in man. Perhaps the following may be considered among the possibilities.

1. The reconversion (via triose) of three-fourths to five-sixths of the lactic acid to glucose and the simultaneous oxidation of the remainder (via pyruvic acid) to  $\text{CO}_2$ , by a "coupled reaction" (83), (141). How the oxidation could facilitate the conversion of lactic acid to glucose is not evident. It is perhaps conceivable that it may result from local change in hydrogen ion concentration. An alkaline reaction appears to favor lactic acid formation, and acid to hinder or limit it.

2. The reconversion of *all* of the lactic acid to glucose or glycogen, with the simultaneous oxidation of glucose or a derivative, (or of fat or other substance).

3. The oxidation of the whole of the lactic acid via pyruvic acid, acetic aldehyde, and  $\text{CO}_2$ , the acetic aldehyde being converted to glucose. This is perhaps less probable, since it would account for the conversion of only two-thirds of the carbon to glucose and one third to  $\text{CO}_2$ , corresponding to a ratio of lactic acid disappearing: oxidized, of 3:1 instead of 4:1 or 6:1. Furthermore the evidence is conflicting as to whether acetic aldehyde is convertible into glucose in the body. Another route, via glyceric acid and glycol aldehyde has been suggested (165). The direct oxidation of lactic acid will be considered again in a later section.

*Blood and kidney tissue.* There is clear evidence that the transformation of glucose to lactic acid takes place also in the blood, the kidney and the liver as well as in muscle; and it is probable that the reaction may take place wherever glucose is metabolized. In 1877 Spiro found lactic acid in blood (186). Levene and Meyer (115) showed that sterile leucocytes suspended in phosphate solution convert added glucose into d-lactic acid. Although the amount of lactic acid formed was less than the amount of glucose which had disappeared, later experiments indicated no evidence of oxidation. Dilution with water, or the presence of toluol prevented the formation of lactic acid.

The amount of lactic acid in whole blood increases on standing (70) and with the increase in lactic acid there has been observed a corresponding decrease in glucose (106), (107). This conversion of blood sugar into d-lactic acid appears to be the explanation of blood "glycolysis" (131) and is brought about by the cells and not by plasma (156). Sterile kidney tissue in phosphate solution and in absence of antiseptics, converts glucose into d-lactic acid, but in the presence of toluol the conversion does not take place (117).

*Liver perfusion.* Much valuable evidence on the reactions between glucose and lactic acid has resulted from the artificial perfusion of

excised liver by a technique developed especially by Embden. By this method of investigation also it has been demonstrated that the reaction  $\text{glucose} \rightleftharpoons \text{lactic acid}$  may proceed in either direction under different circumstances.

With livers containing glycogen there is an increase of glucose (108), and of lactic acid (38), (50) in the perfusion fluid. But with livers rendered glycogen-free by producing strychnine convulsions in the animals, lactic acid is not formed, unless glucose, fructose, alanine, or glycerol is added to the blood. The addition of arabinose or inosite is without effect (50), (53). These results show that glycogen or glucose is the source of the lactic acid.

The reverse transformation, lactic acid to glucose, takes place on perfusing glycogen-free livers, either normal or diabetic. Embden (38) found an increase of blood sugar with livers rendered glycogen-free by strychnine convulsions, while Embden and Kraus (50) found under the same conditions a decrease of lactic acid. And Embden and Isaac (48) showed that the loss of lactic acid closely parallels the gain of glucose, thus indicating the source of the glucose and showing an approximately *quantitative conversion*.

With livers of *depancreatized* dogs, Lattes (110) found a marked *increase* of blood sugar after perfusion, and Embden and Isaac showed that the loss of lactic acid with such livers almost exactly equals the gain of sugar, again indicating a quantitative conversion. Added glucose did *not* give rise to lactic acid with diabetic livers, although it is so converted by normal livers. Barrensechen (10) reported that diabetic livers on perfusion do not form glycogen from added hexoses, under conditions in which glycogen is formed by the normal liver. These results appear to show that the liver of the normal dog, if glycogen be abundant, converts it to glucose and to lactic acid; or when the glycogen store is low, it can reverse the process and form glycogen from added glucose or lactic acid. But the liver of the diabetic dog has lost the power to carry the reactions, glucose to lactic acid, and glucose to glycogen, but retains the power to convert lactic acid to glucose. The conditions may be represented as follows:

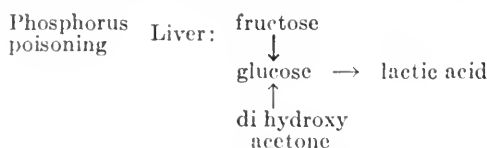
Normal liver: Glycogen  $\longleftrightarrow$  glucose  $\longleftrightarrow$  lactic acid

Diabetic liver: Glycogen  $\longrightarrow$  glucose  $\longleftarrow$  lactic acid

These findings recall the facts that the diabetic animal or human subject converts lactic acid and many other substances into glucose, though he does not readily form lactic acid nor store glycogen. Wood-

yatt (201) reports that the muscle of a human diabetic does not develop lactic acid in rigor, as does normal muscle. Similar, though less striking results have been reported by Von Furth (73). But Forschbach (63a) found that the muscle of a depancreatized dog contained a normal amount of lactic acid. And Parnas (164) finds that the muscle of depancreatized frogs does not differ from normal frog muscle in its formation and oxidation of lactic acid on contraction, and suggests that diabetes does not interfere with the consumption of carbohydrate for muscular work. This conclusion can hardly be accepted for man or warm blooded animals.

The livers of animals poisoned with phosphorus, in contrast with diabetic livers, on perfusion are found to possess the power to form lactic acid from glucose or fructose, but have lost the power to accomplish the reverse change of lactic acid to glucose. They do however form glucose from dihydroxy acetone (101). The defect thus would appear to be between lactic acid and triose. Fructose is converted to glucose by phosphorus livers as well as by normal livers.



Livers of animals poisoned with both phosphorus and phlorhizin form no glucose from lactic acid. This result is surprising in view of the fact that phlorhizin injection (and glycosuria) inhibits lactic acid excretion by phosphorus poisoned animals (134). E. Neubauer (148) claims that rabbits poisoned with phosphorus can form glycogen from fructose but not from glucose.

THE INTERMEDIATE PRECURSORS OF GLUCOSE  $\rightleftharpoons$  LACTIC ACID IN VIVO. The evidence cited shows that each of the above-named substances may be converted into the other, in the body as a whole and in isolated organs or tissues. The conversion is the result of a series of transformations, the analysis of which has been attempted by determining what possible intermediates or related substances give rise to either glucose or lactic acid when fed to animals or when added to isolated organs or tissues or to perfusion fluids. The following facts appear to indicate the same intermediates in the *in vivo* reaction as in the *in vitro* formation of lactic acid from glucose by alkali.

d-l Glyceraldehyde may be oxidized in the body (150), is destroyed by liver tissue (184), is converted into d-glucose when fed to phlorhizin-

ized dogs (177), forms glycogen on perfusion of tortoise livers (162), forms d-glucose and d-sorbose on perfusion of dog liver (56), and is converted into d-l and l-lactic acid by washed blood corpuscles and by perfusion of livers of fasting dogs (42). It may therefore go in either direction: glucose  $\leftarrow$  glyceric aldehyde  $\rightarrow$  lactic acid. Similarly, dihydroxy acetone is converted to d-glucose when injected into phlorhizinized dogs (172), and by perfusion of dog liver it gives both d-glucose (56) and d-l lactic acid (42). On standing with washed blood cells of the dog it is converted to d-l lactic acid less readily than is d-l glyceric aldehyde, but pig blood corpuscles form from it d-lactic acid (125), (78).

Methyl glyoxal is likewise converted into glucose by the phlorhizinized diabetic dog (30) and into d-l-lactic acid by many hashed tissues (by the enzyme "glyoxalase"), (28), (29), (151a), on liver perfusion and by sterile leucocytes and kidney tissue (116).

As emphasized especially by Dakin and Dudley, Levene and by Neuberg, methyl glyoxal must, in the light of the above facts, be regarded as an intermediate between glucose and lactic acid. In view of the wide occurrence and great activity of the enzyme "glyoxalase" there is good reason to believe that it is the immediate precursor of lactic acid *in vivo*, as it doubtless is in the formation of lactic acid from sugar *in vitro*. Dakin has pointed out that the undoubted conversion of l-lactic acid into d-glucose seems to necessitate its passage through an optically inactive intermediate, and that this is probably methyl glyoxal or dihydroxy acetone. The opposite opinion advanced by Embden, Baldes and Schmitz (42) that methyl glyoxal cannot be an intermediate because its molecule contains no asymmetric carbon atom, can scarcely be accepted in view of the wide distribution of "glyoxalase" and of the undoubted conversion of l-lactic acid and of many racemic compounds into d-glucose. Neuberg (152) points to the possible existence of forms of methyl glyoxal with optically active carbon atoms, and suggests schemes by which molecular asymmetry might be conferred by it as an intermediate.

There is therefore no obstacle to the belief that the three substances, glyceric aldehyde, dihydroxy acetone and methyl glyoxal (together with the hexose phosphate, "lactacidogen") represent the main intermediates between glycogen or glucose and lactic acid.

Although the reactions in the direction of lactic acid have close analogies in the *in vitro* behavior of glucose, the reverse reactions, lactic acid to glucose, which appear to occur only under the influence of living cells and *in the presence of oxygen*, are difficult to visualize.

The apparent necessity of oxygen suggests that the path may be by way of an oxidation of lactic acid. Such a conception has been advanced by Parnas and Baer (165) who propose a series of reactions by which three molecules of lactic acid yield progressively glyceric acid,  $\beta$ -hydroxy pyruvic acid and glycol aldehyde, the latter condensing to a molecule of glucose. As evidence for their scheme they find an increase of glycogen in tortoise liver after perfusion with glycol aldehyde (but not with glycollic or glyoxylic acids) and a conversion of glyceric acid, lactic acid, glycol, and glycol aldehyde into glucose by phlorhizinized rabbits. Barrensheen also (10) observed the formation of glucose from glycol aldehyde, glycerol, glyceric acid and lactic acid on perfusion of livers of phlorhizinized dogs. However, Baldes and Silberstein (8) in similar experiments were unable to show the formation of sugar from glyceric acid or glycol aldehyde, and the scheme of Parnas and Baer (165) has not been generally accepted (27, p. 116). It appears, nevertheless, that the two-carbon glycol aldehyde, if formed in the body, may be converted into glucose. Although the observation by Mayer (136) of glycosuria after its administration to rabbits may be of doubtful value the experiments of Sansum and Woodyatt (178), Cremer (25) and Greenwald (76) with phlorhizinized dogs, if not decisive, appear to show the formation of glucose from the injection of glycol aldehyde. Neither glyoxal nor glycollic acid, into which the former is converted by surviving liver (31), are converted to glucose (76). It seems, therefore, that glycol aldehyde can form glucose only by direct condensation before oxidation or action by "aldehydemutase" (161). It will be recalled that in alkaline solution glycol aldehyde polymerizes to tetroses (60) which then dissociate with the formation of lactic and other acids (66).

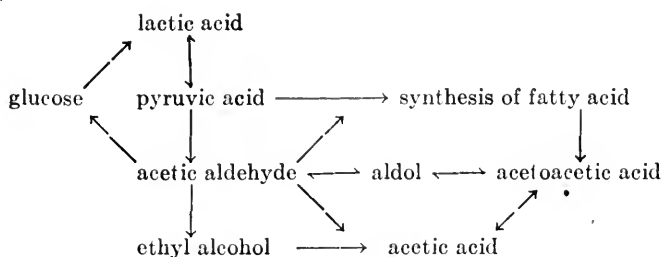
Another possible pathway of oxidative conversion of lactic acid to glucose, via pyruvic acid and acetic aldehyde, the latter condensing, with oxidation to glucose, has been already noted. (P. 414 see also p. 422.) Opposed to such explanations is the fact that diabetic animals appear to convert all of the carbon of lactic acid into glucose, and if this be true a direct conversion, without loss of carbon by oxidation, is of course demanded. Glycid and acetole are not convertible into glucose (77).

It appears at present that the most probable course from lactic acid to glucose is passage through almost if not quite the same intermediates which precede the formation of lactic acid, i.e., by a series of *reversible reactions*; though the apparent oxidative character of the reaction

suggests caution in the adoption of this conclusion. The step from lactic acid to methyl glyoxal has an *in vitro* parallel in the formation of methyl glyoxal in lactic acid solutions (30). The next step, from methyl glyoxal to triose has not yet been accomplished *in vitro*, but the third step, triose to hexose was demonstrated by the well known synthesis of fructose from glycerose by Fischer.

**THE FURTHER FATE OF LACTIC ACID.** Of the two possibilities for the disposal of lactic acid one has just been considered—its direct reconversion to glucose and glycogen via methyl glyoxal and triose. The other is its direct oxidation. The first seems very probably to occur, and the second, oxidation by way of pyruvic acid and acetic aldehyde, represents what appears to be the prevailing view as to the main path of carbohydrate oxidation (Neuberg, Embden, Meyerhof).

In 1911 Neuberg (153) discovered that yeasts decompose pyruvic acid into acetic aldehyde and  $\text{CO}_2$ ; and in a series of later investigations with various collaborators he has admirably demonstrated the fundamental importance of these substances in the intermediary metabolism of glucose in fermentations. There has gradually been accumulated a train of evidence which has been interpreted in support of the idea that glucose metabolism in the animal body follows much the same path as in fermentation and that pyruvic acid and acetic aldehyde are in the animal body also obligate intermediates. These two substances are regarded not only as steps in the oxidation of glucose, and in the oxidative reformation of glucose from lactic acid, but as a bridge by which glucose passes to fatty acid or to acetoacetic acid, or vice versa. The following diagram represents the possible reactions under consideration.



Without questioning the experimental results the reviewer finds it difficult to accept some of these conclusions. Although on oxidation by hydrogen peroxide *in vitro*, lactic acid yields pyruvic acid (92) no *direct* evidence appears to be available as to the products of lactic acid oxidation in the body. That pyruvic acid is the product is inferred

from the fact that its decomposition product, acetic aldehyde has been found, and from the fact that the reverse reaction, its reduction to lactic, is established. Tschernorutzki (191) found that on digestion with muscle or liver hash pyruvic acid disappears, but he was unable to find acetic aldehyde. By liver perfusion, however, Embden, and Oppenheimer (53) observed on adding salts of pyruvic acid an increased formation of acetoacetic acid, which they interpreted as being due to preliminary formation of acetic aldehyde and its condensation to aldol. Friedmann had earlier found that an increase of acetoacetic acid resulted from perfusion of liver with acetic aldehyde. Although the amounts of acetone (from acetoacetic acid) found by Embden and Oppenheimer were neither large, nor constant, the authors put forward the view (53), (54) that glucose  $\rightarrow$  lactic acid,  $\rightarrow$  pyruvic acid,  $\rightarrow$  acetaldehyde  $\rightarrow$  acetoacetic acid,  $\rightarrow$  acetic acid, is the main path of glucose metabolism.

If lactic acid (or glycogen) is the source of pyruvic acid and if the latter gives rise to acetic aldehyde, aldol and acetoacetic acid, as depicted in the diagram, it might be expected that with increasing formation of lactic acid there would be increasing amounts of acetone. But the reverse appears to be the case. On perfusion of glycogen-rich livers, (or normal glycogen-free livers with added glucose or fructose), there is much lactic acid formed, but little or no acetone; while with diabetic livers, which convert lactic acid almost quantitatively to glucose, large amount of acetoacetic acid appear (48). This would seem to prove that the source of the acetoacetic acid (and of pyruvic acid and acetic aldehyde) is not lactic acid. If lactic acid is oxidized its products must *inhibit* the formation of acetoacetic acid.

The evidence is more satisfactory for the reverse conversion of pyruvic acid (whatever its origin) into lactic acid and glucose. Mayer (137) found lactic acid in the urine of rabbits after giving pyruvic acid. Embden and Oppenheimer found a very marked increase of d-lactic acid on perfusion of surviving dogs' liver with blood containing salts of pyruvic acid, and interpreted the earlier observation (50) of lactic acid from perfusion with alanine as being due to the intermediate production of pyruvic acid (149). It is somewhat surprising that on perfusion of livers of phlorhizinized dogs Baldes and Silberstein (8) and Barrescheen (10) could find no evidence of glucose formation from pyruvic acid (or from alanine or serine). When administered to phlorhizinized dogs, pyruvic acid is, in part at least, converted to glucose, presumably by reduction to lactic acid (169), (170), (32),

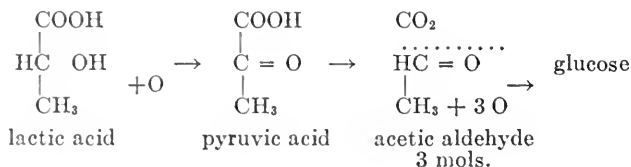
(25). This conversion is less constant and complete than is observed with lactic acid, and it is perhaps possible that this is to be explained by a dual path, lactic acid,  $\leftarrow$  pyruvic acid,  $\rightarrow$  acetic aldehyde. Levene and Meyer (119) were unable to observe any action on pyruvic acid by sterile leucocytes, under conditions where a formation of lactic acid from glucose was found to take place.

Other more direct evidence has recently appeared in support of the view that *acetic aldehyde* is an intermediate in animal metabolism.

Stepp and co-workers (189) find and identify traces of acetic aldehyde in the blood and urine of diabetic subjects. In view of the observation (67), that acetaldehyde is converted to acetoacetic acid on liver perfusion, they assume the aldehyde to be a precursor via aldol, of the "acetone bodies." But Fricke (69) was able to find only doubtful traces of aldol from 50 liters of diabetic urine. Hirsch (90) shows that on aerating hashed frog muscle and fish muscle in the presence of "dime-don," acetaldehyde is formed (73 mgm. from 500 grams muscle). Traces were obtained also from fresh frog muscle, the amount not being increased on aeration except in the presence of the "dime-don" so successfully used by Neuberg and Reinfurth (155) in yeast fermentation. The origin of the acetaldehyde in these experiments was supposed to be pyruvic acid, derived from the oxidation of lactic acid, and thus from carbohydrate, though direct evidence for the latter assumptions is lacking. It is perhaps equally probable that the source is pyruvic acid formed from amino-acid (149). Until a decision on these points can be reached, judgment as to the significance of these observations must be withheld.

Concerning the behavior of acetic aldehyde in the body, the following results have been reported. Batelli and Stern (11) find that tissues convert it into alcohol and acetic acid (41), which action according to Parnas (161) is accomplished by enzymes which he terms "aldehyde mutase." This may be the origin of the small amounts of alcohol found in animal tissues (A. E. Taylor, 1913). Both acetic acid (124) and ethyl alcohol (138) as well as acetic aldehyde (67) are claimed to give rise to acetoacetic acid on liver perfusion. Neither acetic acid nor alcohol are ketogenic in the body as a whole, and the above conclusions from liver perfusion are open to question. Neither acetic acid nor ethyl alcohol are convertible into glucose. On giving acetic aldehyde subcutaneously to phlorhizinized dogs, Ringer and Frankel (172) found an increased glucose excretion, although Sansum and Wood-yatt (176) after similar experiments concluded that a formation of

glucose from acetic aldehyde does not take place. Lusk (128) states that S. R. Benedict has found the conversion of acetaldehyde to glucose to be complete in the phlorhizinized dog. If this be true, it perhaps constitutes another path for the oxidative conversion of lactic acid to glucose considered in an earlier section.



Ringer and Frankel found acetaldehyde to be antiketogenic, which accords with its conversion to glucose, but is contrary to its apparent conversion into acetoacetic acid on liver perfusion (67). Fricke (69) quotes Reizenstein as showing that acetaldehyde is not easily oxidized in the body, a considerable fraction being excreted in the urine after its administration. If this be true, it would seem unlikely that such large amounts of acetaldehyde are formed as must occur if it is an intermediate in glucose oxidation, or in the conversion of lactic acid to glucose.

Although a decision as to the origin of acetic aldehyde and the extent of its rôle in metabolism must await more conclusive evidence, it seems to the reviewer probable that it has no direct relation to glucose metabolism. If formed it would give rise, at least in part, to acetoacetic acid (67); but the oxidation of glucose *prevents* ketosis. In view of the definite and quantitative relations which appear to exist between the opposing metabolism of ketogenic and antiketogenic substances (Shaffer) (182) one is inclined to doubt whether any intermediate of antiketogenic glucose can *directly* give rise to ketogenic molecules, or vice versa. The conversion of carbohydrate into fat is, of course, an example of such transformation, but this presumably occurs only with carbohydrate plethora. Although on theoretical grounds one might expect the reverse transformation, there is no acceptable evidence that such actually occurs in the body. When the amount of carbohydrate available is small and the need for glucose correspondingly great, a transformation of ketogenic to antiketogenic substance in either direction does not appear to take place (182). It must be noted however that the opposite conclusion is reached in a recent review by Geelmuyden (74).

The argument that acetic aldehyde is formed in the animal body from glucose via lactic and pyruvic acids, is converted by synthesis back to glucose, is converted to acetoacetic acid or formed from it, and is thus a bridge between carbohydrate and fat, is a very attractive hypothesis, but one which the reviewer regards as fallacious.

*The conversion of carbohydrate into fat.* In connection with the rôle of pyruvic acid and acetic aldehyde, it should be noted that each of these substances has been regarded as the probable intermediate in the synthesis of fatty acid from glucose. Based on a suggestion of Nencki, Magnus-Levy (133) proposed a progressive aldol condensation of acetic aldehyde formed from *lactic acid*, followed by reduction of the  $\beta$ -carbon alcohol groups, as the sequence in the formation of straight chain fatty acids.

A somewhat similar hypothesis was developed by Smedley (185) who showed that a repeated condensation of pyruvic acid with loss of  $\text{CO}_2$  and reduction may result in straight chain fatty acids with an even number of carbon atoms.

Some such process is probably the explanation of fatty acid synthesis, but in the absence of direct evidence, they can be regarded only as speculative hypotheses. No other scheme seems to have been proposed.

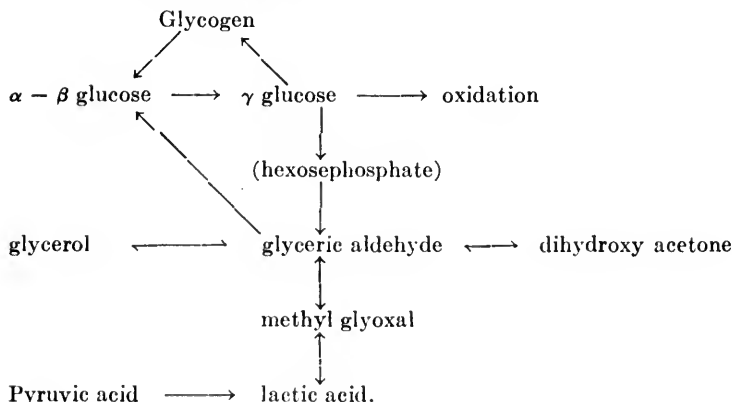
THE PATH OF GLUCOSE OXIDATION IN THE ANIMAL BODY. It has been noted above that there are difficulties in the acceptance of the view that glucose is *oxidized* via lactic and pyruvic acids and acetaldehyde. A summary of the main points opposing such a course may be made as follows.

Although the conversion,  $\text{glucose} \rightleftharpoons \text{lactic acid}$ , takes place in either direction in the body as a whole, or in isolated muscle or liver, the *preponderating* reaction in the presence of oxygen, seems to be in the direction,  $\text{glucose} \leftarrow \text{lactic acid}$ . Lactic acid appears only as the result of muscular activity or asphyxia, and when it disappears from isolated muscle or during liver perfusion, it is *chiefly* converted into glucose or glycogen. There is no convincing evidence of its direct oxidation to pyruvic acid or other products, though the possibility that such occurs cannot be denied. When pyruvic acid is introduced into the body or used in liver perfusion its *main* fate seems to be the conversion to glucose or lactic acid.

According to present views, the sudden, explosive formation of lactic acid is the essential agent causing muscular contraction (93), (87), (141). But during muscular work on low carbohydrate diets, the respiratory quotients indicate that the substance oxidized is fat. (See for example the careful work of Krogh and Lindhard (109) also Eckert (36).) Under such conditions presumably lactic acid is formed, but it cannot be the substance *burned*: unless it be supposed that fat, the ultimate source of the energy, is converted into glucose or other precursor of lactic acid in the course of oxidation. Although held by some authors, (see review by Geelmuyden) (74) the best evidence seems to be contrary to the latter view. If glucose or glycogen is the only source of lactic acid, and if the latter is necessary to muscular contraction its *conservation* under such circumstances would seem to be essential.

In the diabetic state, with the loss of the power to oxidize glucose, there is also the inability to burn lactic acid and any of its precursors or intermediates, proved by the fact that all are completely and quantitatively converted to glucose. The livers (48) and muscles (73), (201) of diabetic animals appear to have lost the power to form lactic acid from glucose which suggests that the diabetic defect is in *the formation of some lactic acid precursor from ordinary glucose*, and since lactic acid itself (or other intermediates) as well as glucose is not burned, one must suppose either an *additional* defect preventing lactic acid oxidation, or assume that it is not directly oxidized even by the normal, but must be first converted to glucose. The latter alternative is the simpler explanation. Recent evidence has been cited which seems to indicate that the diabetic defect is located in the step between ordinary  $\alpha$ - and  $\beta$ -glucose and a tautomeric form,  $\gamma$ -glucose, which it may be imagined is an essential step in the transformations to glycogen, or to methyl glyoxal and lactic acid, or to oxidation.

This conception appears to remove another fundamental difficulty. According to the results cited from Embden's laboratory, the liver of the depancreatized dogs have lost the power to form lactic acid from glucose, but retain the ability to perform the reverse change, possessed alike by the normal and diabetic intact animal (48). It is difficult to interpret these results on the basis of a single reversible reaction the same throughout in both directions. But if the reactions be looked upon as in a cycle, at one stage of which the pancreatic hormone is necessary, the experimental findings become more nearly intelligible. The following crude outline represents this idea, and appears to the reviewer best to coincide with existing facts.



The diagram is intended to indicate that the synthesis of glucose from lactic acid and intermediates gives ordinary  $\alpha$ - $\beta$ -glucose, which must be transformed into a reactive isomeric (ethylenic oxide?) formed by the intervention of pancreas hormone (9), (17), (34) before the reverse change to lactic acid can occur; and that the formation of lactic acid is a side reaction, asphyxial in character, and concerned with muscular contraction and perhaps with other phenomena, but probably not with glucose *oxidation*. Pyruvic acid is represented as undergoing reduction to lactic acid; the reverse reaction seems doubtful.

If lactic acid be rejected as the path of glucose oxidation, at what other intermediate does its oxidation start? The same sort of objections seem applicable to methyl glyoxal and to glyceric aldehyde. Both are converted into d-glucose by the diabetic organism, and this fact may be interpreted either by supposing the diabetic defect to prevent their direct oxidation, or that their conversion to glucose is the normal reaction, and that it is glucose which fails of further utilization. In view of the discovery by Dakin and Dudley (29) that pancreas extracts inhibit the conversion of glyoxals to hydroxy acids by other tissues, and the statement by Hopkins (93) that pancreas extract slows the rate of lactic acid production in hashed muscle, it is tempting to pick the very reactive methyl glyoxal as the substance oxidized.

An observation by Sansum and Woodyatt (178) casts doubt upon glyceric aldehyde. They find that the tolerance of d-glyceric aldehyde, or the rate at which it may be injected intravenously without triosuria, is only one-sixth the rate at which glucose is *burned* in the body, and that the combustion of glucose cannot, therefore, take place via glyceric aldehyde. Assuming glyceric aldehyde to be a precursor of methyl

glyoxal and lactic acid, Woodyatt's argument would seem to apply with equal force to the last two substances and to the further products of lactic acid oxidation as well as to glyceric aldehyde. It would seem, therefore, that by exclusion we are forced to turn to some form of glucose itself as the substance from which its oxidation begins.

This conclusion is supported also by the behavior of glucose (carbohydrate) in inhibiting by its oxidation the appearance of ketosis. A discussion of this subject, the explanation and mechanism of "antiketogenesis," is beyond the scope of this review, but it may be noted that its recent analysis (182) appears to show that the avoidance of the appearance of acetoacetic acid (and of acetone and hydroxy butyric acid) in the body is due to its combining with a product of the oxidation of glucose, which reaction allows its oxidation. When glucose is not being oxidized and its "ketolytic" oxidation product is not being formed in sufficient amounts, in relation to the rate of formation of acetoacetic acid, the latter accumulates with resulting ketosis. The oxidation of glucose in alkaline solution *in vitro* accomplishes the oxidation of acetoacetic acid in a manner which appears to be quite analogous to the reaction in the body. In the human subject the maximum antiketogenic effect of glucose corresponds to approximately 2 molecules of acetoacetic acid for 1 of glucose, which is also the ratio of glucose to keto-acid in the *in vitro* reaction. But *in vitro*, each molecule of glyceric aldehyde, glycol aldehyde (their oxidation products) or glyoxal likewise reacts with two of acetoacetic acid. If in the body, (or *in vitro*) glucose were first dissociated into two molecules of glyceric aldehyde, we should expect that under favorable conditions it would accomplish the oxidation of  $(2 \times 2)$  or 4 molecules of keto-acid. But this would be *twice* the maximum antiketogenic effect of glucose which has so far been observed. According to this line of argument *it must therefore be concluded that the six carbon glucose is probably oxidized to a single molecule of ketolytic substance without previous splitting to triose.*

What the first oxidation products of glucose may be are quite unknown. Levene and Meyer could find no evidence that glucosone is acted upon by tissues (120). In view of the apparent similarity between its oxidation in the body and in alkaline solution *in vitro*, further study of the latter may be of assistance in solving the problems.

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## BACTERIAL METABOLISM

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INTRODUCTION. "In the study of the microscopic forms known as bacteria we have what might fitly be called the focal point of the various branches of biological science. Though their investigation may require careful morphological researches, yet the unmistakable monotony of form, combined with a considerable variation of physiological activity, has compelled the bacteriologist to pay much attention to means by which such physiological variations may be more or less accurately registered in order that they may serve as a supplementary basis for classification. Again, with the unicellular organisms the manifestations of cell activity become the most important phenomena for study. These manifestations bring together the fields of physiology and chemistry and make Bacteriology in one sense a branch of physiological chemistry" (1).

Three decades have passed since this prophetic assertion of the interdependence and mutual relations of the fundamental dynamic biological sciences to the solution of the manifestations of cellular activity was published, yet even today its full import is foreshadowed rather than realized.

These three decades, nevertheless, have been replete with contributions to the well-being of mankind. They have witnessed the maturation of the germ theory of disease, the rise and development of a new science, immunology, and the first great accomplishments in the field of preventive medicine. The widening of the frontiers of knowledge in physiology and pathology have influenced profoundly the advance of curative medicine.

The pioneer work in the accurate measurement of the interchange of living cells with their environment belongs also to this period, and the widespread transition from the static or morphologic contemplation of biologic science to the dynamic or causative aspect has revealed new and fruitful fields for study.

Bacteriology also has passed through this transitional period from morphology and classification to an inquiring into the causation and effect of microbic activity. The search for curative serums, antitoxins, vaccines, and the response of the host to these remedial agents, has impelled investigators in many fields to take up anew the study of cellular activity and the results thereof. It is not without significance that many of the requisite procedures for this study have been developed by the biochemists.

**PART I. THE BACTERIAL CELL.** Bacteria are among the smallest of known living things. The phenomena of life are revealed in their lowest terms in this group of organisms. All of their vital functions are consummated in single cells, so minute that fully twenty-five thousand of average size, laid side by side, would scarcely span the distance of an inch. Bacteria possess no morphologically definable nucleus,<sup>1</sup> they are devoid of chlorophyll or other photodynamic pigment, and they reproduce by simple fission, the resulting individuals being of approximately equal size. This process of transverse fission of a single sexless unit is the simplest method of reproduction thus far revealed in nature. It may be very rapid. For example, cholera vibrios placed in a very favorable environment may divide individually into two daughter vibrios every fifteen minutes for limited periods of time. The *theoretical* twenty-four hour progeny of a single cholera vibrio, therefore (ninety-six generations), would be  $2^{96}$ , or nearly  $8 \times 10^{28}$ , a truly prodigious number. Fortunately, many natural barriers, as mutual antagonism, exhaustion of food and accumulation of waste products, restrain the multiplication of the microbes after the first few hours' growth and keep the progeny within endurable limits. Nevertheless, the rapid increase of bacteria in short periods of time is no inconsiderable factor in determining the rate and extent of the changes they induce in their nutritive environment.

Another noteworthy characteristic of the bacterial cell is its disproportionately large ratio of surface area to weight or volume. Thus, a typhoid bacillus is a microbe of average size. Its volume is approximately 0.000,000,002 cc. and its weight about 0.000,000,002 mgm. (2). Its surface area, however, is nearly 0.000,01 sq. mm. In no other group of known living things, except possibly the filterable viruses, is this ratio of surface to volume exceeded, or even equalled.

<sup>1</sup> The bacterial cell, chemically considered, is relatively rich in nuclear substance, however.

Inasmuch as the energy requirements of living things in general are determined largely by the ratio of their surface to their volume, this large surface-volume ratio characteristic of bacteria furnishes a background for the well attested magnitude of microbial interchange with their nutritive environment, notwithstanding their minuteness. The two factors—rapid multiplication and large surface to volume ratio—underlie the phenomena of bacterial nutrition.

PART II. BACTERIAL METABOLISM. *a. General considerations:* Bacteria and animals differ from plants in that they possess no chlorophyll or other photodynamic pigment. Chlorophyll is a transformer of solar energy into growth energy. In virtue of this transformed energy the plant weaves the structural elements—nitrates, carbon dioxide, water and salts—into the complex proteins which form the living substance of the plant tissues. The architectural design of these organic complexes resides in the heredity of the plant itself, which guides the life elements into the complex specificity of the plant protoplasm.

The absence of chlorophyll or other photodynamic pigment from the bacterial substance makes it quite evident that the driving force of microbial existence must come directly or indirectly from energy-producing substances formed by green plants. Stated differently, bacteria require at least some preformed food in their dietary; they are essentially transformers, not accumulators of energy. The utilization of food by bacteria is distributed between two distinct phases of their life history, which overlap somewhat in point of time. The first is the constructive or anabolic phase, in which the parent cell, after enlarging somewhat, divides into two daughter cells, each of approximately equal size. The anabolic phase, chemically considered, is one in which the hereditary chemical complex of the protoplasm of the bacterial cell, together with the requisite enzymes and other armamentaria, is elaborated from simpler organic substances obtained from the nutritive environment.

The second, or catabolic phase, is the energy phase, in which the morphologically mature cell performs its peculiar and specific functions as a distinct biological entity. The requisite energy for the performance of these specific activities is derived from the intracellular degradation of suitable organic substances absorbed from the nutritive environment by the mature microbe. The totality of this interchange by bacteria with their nutritive environment, through which they obtain the requisite substances for their structural and energy requirements, is very properly designated bacterial metabolism. The chemistry of bacterial metabolism is the adumbration of bacterial activity.

*b. The anabolic phase of bacterial metabolism.* Chemical analysis of the bacterial cell reveals the presence of nitrogen, carbon, hydrogen, oxygen, together with smaller amounts of phosphorus and inorganic salts, in about the same proportions as those found in other typical cells of the animal and plant kingdoms. Phosphorus, however, is disproportionately abundant; this element, as phosphoric acid, forms the inorganic basis for nucleo protein. Nucleo protein is chemically prominent in the bacterial substance, even though not demonstrable by ordinary nuclear strains. It follows that bacterial growth depends upon the availability of these elements, both in amount and in appropriate combination.

The most significant of the essential vital structural chemical elements is nitrogen. It is strikingly inert, yet in combination with hydrogen as amino nitrogen it is the very cornerstone of life. The amino acids, of which some nineteen are known, united in a multitude of combinations, form the specific protein of all living things. With the possible exception of the nitrogen-fixing microbes and the nitrifying organisms, bacteria require, or utilize more readily, amino (or ammonia) nitrogen for their structural needs. The complexity of nitrogen combination required varies from the simplest peptids to the most complex of proteins. A few types can utilize very simple nitrogenous compounds. The great majority thrive in media containing polypeptids, or peptones. A small group of the most highly parasitized microbes, as for example the gonococcus, require, or thrive best, in media containing protein but little changed from the state in which it exists in the human or the animal body. No known microbes will develop in media from which nitrogen is excluded.

Chemically considered, the anabolic or structural phase of bacterial metabolism is a series of hydrogenic condensations in which amino acids, or complexes thereof, together with carbon, hydrogen, oxygen and inorganic salts, are woven by the microbe into the complex specific nitrogenous substance or protoplasm which determines the distinctive chemical architecture of the microbial species. Simpler carbohydrates are similarly united to form starchlike compounds, and fatty acids and glycerol, or other alcohols are united to form waxes and fats.

The actual amount of nitrogenous compounds, carbohydrates and fats entering into even very large numbers of bacteria, is small indeed. Fully fifteen millions of million typhoid bacilli would scarcely balance an ounce weight. About 85 per cent of this microbial substance is water. Even with a liberal allowance for wastes, a very small quantity

of nitrogenous substance would suffice to furnish the nitrogen of this mass of typhoid bacilli.

The anabolic or structural phase of microbial life, therefore, insofar as it can be differentiated from the energy phase, is relatively inconspicuous both with reference to the amount of chemical interchange with the nutritive environment, and with regard to the nature of the products resulting therefrom.<sup>2</sup>

*c. The catabolic or energy phase of bacterial metabolism.* The part played by bacteria in nature, their specificity of action in other words, depends very largely upon the character of the carbonaceous substances from which they obtain their energy. The amount of substance required to furnish this energy far exceeds that necessary to provide the essential structural material. The physical basis for this marked disproportion between structural and energy requirements resides largely, but not wholly, in the large surface area of the individual microbe in proportion to its volume or weight.

Two great classes of energy-containing substances are generally available for microbial catabolism, namely, the carbohydrates, and the proteins or their derivatives. Oxidizable carbon is contained in each, but the substances resulting from the respective intracellular vital combustions of this carbon are, or may be, widely different. Thus the same organism, utilizing the carbon of a carbohydrate for its energy on the one hand, or the carbon of a peptone on the other hand, may be a veritable Dr. Jekyll or a Mr. Hyde in so far as the results of its activity are concerned. A few examples selected from a multitude of well authenticated instances of this important phenomenon will be illuminating.

The diphtheria bacillus, growing in a broth medium containing peptone and meat extractives, but no utilizable carbohydrate, produces that potent specific soluble toxin known as the diphtheria toxin. The colon bacillus cultivated in the same medium forms indol from the amino acid tryptophan. *Bacillus proteus* elaborates a soluble proteolytic enzyme under similar conditions. The Shiga dysentery bacillus generates a soluble poison from the same ingredients, which is said to be unlike that formed by any other microbe. Each organism, so it appears, acts characteristically and differently upon the common

<sup>2</sup> The poisons formed by the group of gas gangrene bacilli may prove to be exceptions. These appear during the first hours of growth and reach a maximum when the culture is multiplying most rapidly. As the culture becomes older, the poisons rapidly disappear (3).

protein constituents of the broth medium, in that the soluble products resulting from its growth are distinctive.

The same holds in the human body, where the protein of the human tissue furnishes the requisite nutriment. The diphtheria bacillus through its exotoxin incites the clinical entity, diphtheria. The Shiga bacillus is the etiologic agent of one type of bacillary dysentery. In like manner *Bacillus coli* forms indol in the alimentary canal from the tryptophan of the ingested protein of the food.

A striking change in the character of the products formed by these microbes takes place if some ordinary glucose is added to the respective cultures before inoculation. It must be remembered that the nitrogenous (protein) constituents of the culture medium remain quantitatively and qualitatively the same; the only change is the mere addition of a small amount of glucose. The diphtheria bacillus does not form the potent, soluble toxin in the glucose-protein medium; it produces lactic acid. The Shiga bacillus fails to make its characteristic poison; it also forms lactic acid. Indol is no longer detectable in the glucose broth culture of the colon bacillus, and no soluble proteolytic enzyme can be detected in the filtrate of the glucose broth culture of *Bacillus proteus*. In place of indol and the enzyme, both the colon bacillus and *Bacillus proteus* cultures contain acidic products, mostly lactic acid.

This remarkable transmutation of the products formed by the four microbes upon the addition of glucose to their nutritive supply is not apparently accompanied by a transformation of their structural phase; indeed, the microbes are qualitatively indistinguishable when they are tested for their specificity with their specific agglutinins, lysins or precipitins.<sup>3</sup> The differences, in fact, are directly attributable to the utilization of carbohydrate in place of protein for their energy requirements.

A mass of information has accumulated which indicates very clearly that utilizable carbohydrate added to cultural media protects or spares the protein of the medium from breakdown for energy among the vast majority of microbes, precisely as carbohydrate protects protein in the animal and the human body.

The effects of adding utilizable carbohydrate to cultural media as exemplified in the four bacteria just mentioned are summarized in the following table:

<sup>3</sup> Simonds (4) has shown, however, that there are quantitative differences in the respective agglutination, precipitation and complement fixation reactions of the plain and glucose cultures.

ORGANISM:	SIGNIFICANT PRODUCT IN PROTEIN MEDIUM:	SIGNIFICANT CHANGE IN THE CARBOHYDRATE-PROTEIN MEDIUM:
Diphtheria bacillus	Soluble diphtheria toxin	Lactic acid. No toxin
Shiga bacillus	Soluble Shiga toxin	Lactic acid. No toxin
Bacillus coli	Indol	Lactic acid. No indol
Bacillus proteus	Soluble proteolytic enzyme	Lactic acid. No enzyme

It follows that the specificity of chemical action of these bacteria depends upon their utilization of protein for energy.

Conversely, when these bacteria are grown in media containing utilizable carbohydrate, they form lactic acid which is the chemical basis of buttermilk. These two generalizations are applicable to all the bacteria parasitic or pathogenic for man thus far studied with the exception of the very few that do not utilize carbohydrate for energy.

PART III. THE QUANTITATIVE MEASURE OF BACTERIAL METABOLISM. The qualitative effects of adding utilizable carbohydrate to cultures of bacteria, illustrated in the preceding section by a very few typical examples, are suggestive of the sparing action of carbohydrates for protein, but they fail to afford satisfying quantitative substantiation of the general principle involved. Physiologists have long been conversant with this same principle as it is manifested in animal and human metabolism. Howell (5) expresses it thus: "The oxidization of the sugar protects the protein of the body." There seems to be little doubt that the sparing action of carbohydrate for protein is a rather general principle of the metabolism of cells in general which require preformed food in their dietary. If such be the case, the study of bacterial metabolism should reveal the pattern upon which the fundamental metabolism of animal and animal-like cells is moulded.

Suitable quantitative chemical methods for the exploration of this field have been perfected by Folin and his associates.<sup>4</sup> It is not without significance in this connection that these procedures were developed by Folin for the study of human metabolism.

The quantitative study of the nitrogenous changes induced in culture media of predetermined composition by many kinds and strains of bacteria has revealed the basis of the phenomena of bacterial metabolism (7) and has indicated the parallelism between microbial (unicellular) and animal and human (polycellular) metabolism. Space does not permit of an extensive discussion of the metabolism of the various

<sup>4</sup> See Kendall (6) for those especially adapted to the study of bacterial metabolism.

types of bacteria, but for convenience of discussion the protocols of the nitrogenous metabolism of two representative organisms are reproduced, namely, *B. typhosus* (8) and *B. proteus* (9):

TABLE 1  
*Bacillus typhosus*, 1

MGM. PER 100 CC.	CONTROL	DAY	PLAIN BROTH	GLUCOSE BROTH
Total nitrogen.....	1.080	1	1.080	1.080
Protein nitrogen.....	0.778		0.800	0.790
Nonprotein nitrogen.....	0.302		0.280	0.290
Polypeptid nitrogen.....	0.210		0.175	0.196
Amino nitrogen.....	0.042		0.048	0.045
Ammonia nitrogen.....	0.050		0.057	0.049
Reaction.....	+0.80		+1.30	+5.10
pH.....	7.2		7.3	5.0
Total nitrogen.....	1.080	4	1.080	1.080
Protein nitrogen.....	0.778		0.811	0.801
Nonprotein nitrogen.....	0.302		0.269	0.279
Polypeptid nitrogen.....	0.210		0.167	0.181
Amino nitrogen.....	0.042		0.044	0.051
Ammonia nitrogen.....	0.050		0.058	0.047
Reaction.....	+0.80		-0.80	+5.40
pH.....	7.2		7.8	5.0
Total nitrogen.....	1.080	7	1.080	1.080
Protein nitrogen.....	0.778		0.923	0.913
Nonprotein nitrogen.....	0.302		0.157	0.167
Polypeptid nitrogen.....	0.210		0.044	0.067
Amino nitrogen.....	0.042		0.043	0.052
Ammonia nitrogen.....	0.050		0.070	0.048
Reaction.....	+0.80		-1.40	+5.80
pH.....	7.2		8.4	5.0
Total nitrogen.....	1.080	10	1.080	1.080
Protein nitrogen.....	0.778		0.868	0.890
Nonprotein nitrogen.....	0.302		0.212	0.190
Polypeptid nitrogen.....	0.210		0.097	0.102
Amino nitrogen.....	0.042		0.038	0.040
Ammonia nitrogen.....	0.050		0.077	0.048
Reaction.....	+0.80		-2.10	+4.20
pH.....	7.2		8.7	5.3

DISCUSSION OF ANALYTICAL TABLES. The methods used in these studies, developed for the most part by Folin and his associates (6),

are of sufficient precision to warrant the supposition that the quantitative changes induced by the bacteria in the nitrogenous constituents of the cultural media furnish a fairly accurate outline of the nitrogenous changes taking place therein during the cultural history of the respective microbes selected for discussion.<sup>5</sup>

*Bacillus typhosus*.<sup>6</sup> The general phenomena indicative of the sparing action of the glucose for the protein are quite clearly discernible. The reaction of the plain, sugar-free medium (plain broth) becomes progressively alkaline, and, also, the hydrogen ion concentration diminishes progressively. Coincidentally, the ammonia detectable in the culture increases from 50 mgm. per 100 cc. medium in the control, to 77 mgm. at the end of ten days' incubation. The increasing alkalinity is presumably attributable to the gradual formation of basic substances. In glucose broth, on the other hand, which has precisely the same nitrogenous composition and reaction, but to which 1 per cent of glucose has been added, the reaction becomes increasingly acid. Both the titratable acid and the hydrogen ion concentration show a rapid and decided increase. This follows from the well attested fact that the products formed from the utilization of sugar for energy are acidic in character and reaction. The free ammonia, however, undergoes practically no change in the glucose broth. Inasmuch as ammonia results for the most part from the deamination of the protein derivatives within the bacterial cell prior to the utilization of the non-nitrogenous residuum of the protein derivative for energy, this disproportion between free ammonia in plain broth and glucose cultures of the organism might be expected.

It is of some interest to determine what type of nitrogenous substance of the culture medium undergoes the most noteworthy change during the growth of the bacteria. A survey of the table shows clearly that the "polypeptid" nitrogen fraction (see reference (6) for detailed explanation) is chiefly altered. The polypeptid nitrogen decreases in amount somewhat more rapidly in the plain broth than the glucose broth during the first week of growth. Then it increases again. This increase is probably due to autolysis of some of the bacteria at the later period, when cultural conditions have become rather unfavorable

<sup>5</sup> For additional studies, see Journ. Infect. Dis., 1922, xxx, no. 2. These appear to be the first complete quantitative investigations of the nitrogenous metabolism of cellular activity recorded.

<sup>6</sup> The analytical figures for the various nitrogen fractions in this and the following tables are expressed in fractions of a gram per 100 cc. culture medium.

for growth. It will be noted that there is a simultaneous increase in the "protein nitrogen" fraction during the first seven days of incubation. This is clearly attributable to the incorporation of some of the "polypeptid nitrogen" into the bodies of the newly formed bacteria. After the seventh day the protein fraction diminishes somewhat, evidence again of autolysis of some of the bacteria. The amino nitrogen does not undergo changes of sufficient magnitude to become of significance.

*Bacillus proteus*: Tables 2 and 3 are reproduced, (pp. 448-449) the first showing the changes induced in plain broth and in the same plain broth to which graded amounts of glucose have been added; the second table shows the changes induced by sterile filtrates of the various broths shown in table 2 in a sterile broth medium.<sup>7</sup> It will be remembered that *Bacillus proteus* forms soluble proteolytic enzymes in media from which considerable amounts of utilizable carbohydrates are excluded. The effect of the increasing amounts of glucose up to 1.5 per cent, at which point the enzyme does not appear in an active state, is clearly indicated. The sparing action of the glucose up to a concentration of 1.5 per cent, which is more than the bacteria can completely oxidize, even in fourteen days, is indicated by the slowness of the action of the enzyme in the higher sugar concentrations during the earlier days of incubation.

The sparing action of the glucose for the protein is also indicated by the very considerable amount of ammonia formed both in the plain broth culture and those containing relatively small amounts of glucose, in contrast to the negligible amount of free ammonia in the 1.5 per cent glucose culture. In the former, the ammonia increase amounts to as much as 200 mgm. per 100 cc. of culture medium after two weeks' incubation. In the latter (1.5 glucose) it amounts to but 6 mgm., a truly striking difference.

Comparing the plain broth culture with the 1.5 glucose broth culture the changes in the "polypeptid nitrogen" are noteworthy. In the former, the polypeptid nitrogen *increases* from 280 mgm. to as much as 963 mgm., whereas in the glucose culture a small *decrease* is clearly discernible. The difference is due to the presence of the soluble enzyme in the plain sugar-free broth culture. This is indicated by the *decrease* in the protein nitrogen fraction from 721 mgm. to 38 mgm. in the plain broth culture, notwithstanding the fact that there has been a considerable amount of protein nitrogen bound up in the bodies of the bacteria. At the end of the seventh day apparently practically

<sup>7</sup> Full details of the experiment will be found in reference (9).

TABLE 2  
*B. proteus*

PROTEUS NO. 1 JUNE 28, 1921 CULTURES	DAY	CON- TROL	PLAIN	PERCENTAGE OF GLUCOSE						
				0.1	0.2	0.3	0.4	0.5	0.75	1.5
Total nitrogen.....	1	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen.....		0.721	0.531	0.564	0.598	0.609	0.665	0.676	0.721	
Nonprotein nitrogen.....		0.280	0.470	0.437	0.403	0.392	0.336	0.325	0.280	
Polypeptid nitrogen.....		0.209	0.389	0.361	0.330	0.319	0.265	0.253	0.213	
Amino nitrogen.....		0.039	0.032	0.028	0.028	0.029	0.030	0.032	0.029	
Ammonia nitrogen.....		0.032	0.049	0.048	0.045	0.044	0.041	0.040	0.038	
Total nitrogen.....	2	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen.....		0.721	0.262	0.195	0.385	0.419	0.453	0.497	0.497	0.743
Nonprotein nitrogen.....		0.280	0.739	0.806	0.616	0.582	0.548	0.504	0.504	0.258
Polypeptid nitrogen.....		0.209	0.638	0.709	0.527	0.415	0.475	0.429	0.434	0.191
Amino nitrogen.....		0.039	0.032	0.030	0.029	0.029	0.028	0.027	0.026	0.030
Ammonia nitrogen.....		0.032	0.069	0.067	0.060	0.048	0.045	0.048	0.044	0.037
Total nitrogen.....	3	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen.....		0.721	0.239	0.239	0.284	0.352	0.430	0.453	0.531	
Nonprotein nitrogen.....		0.280	0.762	0.762	0.717	0.649	0.571	0.548	0.470	
Polypeptid nitrogen.....		0.209	0.647	0.647	0.608	0.536	0.473	0.469	0.400	
Amino nitrogen.....		0.039	0.036	0.036	0.032	0.030	0.047	0.030	0.028	
Ammonia nitrogen.....		0.032	0.089	0.089	0.077	0.056	0.051	0.049	0.042	
Total nitrogen.....	4	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen.....		0.721	0.060	0.073	0.139	0.228	0.262	0.307	0.351	
Nonprotein nitrogen.....		0.280	0.941	0.928	0.862	0.773	0.739	0.694	0.650	
Polypeptid nitrogen.....		0.209	0.796	0.777	0.709	0.696	0.654	0.613	0.571	
Amino nitrogen.....		0.039	0.028	0.035	0.035	0.018	0.035	0.032	0.030	
Ammonia nitrogen.....		0.032	0.117	0.116	0.118	0.059	0.050	0.049	0.049	
Total nitrogen.....	5	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen.....		0.721	0.161	0.127	0.161	0.206	0.206	0.239	0.340	0.822
Nonprotein nitrogen.....		0.280	0.840	0.847	0.840	0.795	0.795	0.762	0.661	0.179
Polypeptid nitrogen.....		0.209	0.663	0.694	0.679	0.654	0.667	0.648	0.567	0.103
Amino nitrogen.....		0.039	0.033	0.031	0.032	0.024	0.025	0.035	0.030	0.039
Ammonia nitrogen.....		0.032	0.144	0.149	0.129	0.117	0.103	0.079	0.064	0.037
Total nitrogen.....	7	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen.....		0.721	0.038	0.094	0.071	0.060	0.083	0.094	0.105	0.732
Nonprotein nitrogen.....		0.280	0.963	0.907	0.930	0.941	0.918	0.907	0.896	0.269
Polypeptid nitrogen.....		0.209	0.696	0.687	0.703	0.757	0.750	0.730	0.722	0.185
Amino nitrogen.....		0.039	0.036	0.025	0.032	0.035	0.042	0.040	0.033	0.044
Ammonia nitrogen.....		0.033	0.204	0.195	0.195	0.149	0.126	0.137	0.141	0.039
Total nitrogen.....	14	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen.....		0.721	0.162	0.151	0.140	0.218	0.151	0.106	0.106	
Nonprotein nitrogen.....		0.280	0.839	0.850	0.861	0.783	0.850	0.895	0.895	
Polypeptid nitrogen.....		0.209	0.587	0.577	0.566	0.511	0.580	0.600	0.624	
Amino nitrogen.....		0.039	0.039	0.026	0.030	0.059	0.057	0.079	0.050	
Ammonia nitrogen.....		0.033	0.213	0.247	0.265	0.213	0.213	0.216	0.221	

TABLE 3  
*B. proteus*

CARBOL GELATIN ENZYME	DAY	CON- TROL	PLAIN	PERCENTAGE OF GLUCOSE						
				0.1	0.2	0.3	0.4	0.5	0.75	1.5
Total nitrogen.....	1	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	
Protein nitrogen.....		0.542	0.325	0.280	0.381	0.392	0.426	0.426	0.471	
Nonprotein nitrogen.....		0.130	0.347	0.392	0.291	0.280	0.246	0.246	0.201	
Polypeptid nitrogen.....		0.102	0.314	0.360	0.257	0.252	0.217	0.217	0.172	
Amino nitrogen.....		0.020	0.025	0.024	0.028	0.022	0.023	0.023	0.023	
Ammonia nitrogen.....		0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.006	
Total nitrogen.....	2	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672
Protein nitrogen.....		0.542	0.235	0.246	0.314	0.403	0.414	0.358	0.370	0.515
Nonprotein nitrogen.....		0.130	0.437	0.426	0.358	0.269	0.258	0.314	0.302	0.157
Polypeptid nitrogen.....		0.098	0.384	0.379	0.314	0.235	0.227	0.285	0.273	0.125
Amino nitrogen.....		0.023	0.044	0.038	0.035	0.026	0.022	0.022	0.020	0.023
Ammonia nitrogen.....		0.009	0.009	0.009	0.008	0.008	0.009	0.007	0.009	0.009
Total nitrogen.....	3	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	
Protein nitrogen.....		0.542	0.078	0.112	0.146	0.202	0.258	0.291	0.347	
Nonprotein nitrogen.....		0.130	0.594	0.560	0.526	0.470	0.414	0.381	0.325	
Polypeptid nitrogen.....		0.097	0.534	0.498	0.475	0.430	0.368	0.344	0.294	
Amino nitrogen.....		0.023	0.048	0.052	0.042	0.031	0.038	0.029	0.023	
Ammonia nitrogen.....		0.010	0.012	0.010	0.009	0.009	0.008	0.008	0.008	
Total nitrogen.....	4	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	
Protein nitrogen.....		1.061	0.389	0.447	0.524	0.515	0.793	0.793	0.804	
Nonprotein nitrogen.....		0.269	0.941	0.883	0.806	0.815	0.537	0.537	0.526	
Polypeptid nitrogen.....		0.119	0.860	0.819	0.737	0.459	0.484	0.484	0.478	
Amino nitrogen.....		0.035	0.065	0.048	0.053	0.041	0.038	0.037	0.032	
Ammonia nitrogen.....		0.015	0.016	0.016	0.016	0.015	0.015	0.016	0.016	
Total nitrogen.....	5	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen.....		1.061	0.322	0.332	0.330	0.390	0.424	0.547	0.670	1.051
Nonprotein nitrogen.....		0.269	1.008	1.008	0.940	0.940	0.906	0.783	0.660	0.279
Polypeptid nitrogen.....		0.211	0.916	0.907	0.844	0.847	0.812	0.700	0.575	0.222
Amino nitrogen.....		0.040	0.073	0.083	0.078	0.075	0.077	0.066	0.067	0.040
Ammonia nitrogen.....		0.018	0.019	0.018	0.018	0.018	0.017	0.017	0.018	0.017
Total nitrogen.....	7	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen.....		1.061	0.367	0.390	0.333	0.547	0.524	0.524	0.524	0.983
Nonprotein nitrogen.....		0.269	0.963	0.940	0.997	0.883	0.806	0.806	0.806	0.347
Polypeptid nitrogen.....		0.205	0.861	0.832	0.884	0.778	0.706	0.706	0.708	0.279
Amino nitrogen.....		0.039	0.076	0.082	0.086	0.081	0.076	0.075	0.073	0.043
Ammonia nitrogen.....		0.025	0.026	0.026	0.027	0.024	0.024	0.025	0.025	0.025
Total nitrogen.....	14	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	
Protein nitrogen.....		1.061	0.413	0.547	0.402	0.402	0.367	0.379	0.469	
Nonprotein nitrogen.....		0.269	0.917	0.883	0.928	0.928	0.963	0.951	0.861	
Polypeptid nitrogen.....		0.191	0.821	0.738	0.820	0.826	0.854	0.847	0.750	
Amino nitrogen.....		0.049	0.067	0.070	0.078	0.073	0.082	0.076	0.082	
Ammonia nitrogen.....		0.029	0.029	0.030	0.030	0.029	0.027	0.028	0.029	

all of the original protein nitrogen has been broken down to the non-protein nitrogen fraction by the enzyme, leaving only that protein locked up in the newly formed bacteria. Strong supportive evidence of this view is found not only in the autolytic changes taking place in the fourteen-day cultures, but also in the 1.5 glucose broth culture, where no enzyme is present. Here the protein fraction *increases* up to the seventh day, while the non-protein fraction *decreases*, precisely as was found to be the case with *Bacillus typhosus*, which forms no soluble enzyme.

Turning to the changes induced by the soluble, bacteria-free enzyme, it will be found that a transfer from the protein to the non-protein nitrogen takes place to a very considerable extent—amounting to a change from 1061 mgm. to 322 mgm. in the fifth day. It should be remembered that only 5 per cent of the normal amount of enzyme found in the cultures of this date has been added to the sterile broth medium; this indicates the activity of the enzyme.

In glucose broth where no enzyme is present, there is no change of any significance in the nitrogenous constituents; this should be expected. One feature deserves special mention. In no instance where the active enzyme (bacteria-free) is added to the sterile medium is there the slightest increase in free ammonia, notwithstanding the great decrease in protein nitrogen. The free ammonia, as indicated above, is associated with the intra-bacterial utilization of the protein for energy. The soluble enzyme is wholly without deaminizing properties.

Not very much "amino nitrogen" is formed by the action of the soluble enzyme upon the protein constituents of the medium. This would suggest that the action of the enzyme is more like that of pepsin than that of trypsin or erepsin. In other words, the soluble enzyme of *Bacillus proteus* does not reduce the protein nitrogen fraction or the polypeptid fraction of the culture medium to the state of single, free amino acids. Rather, there is a cleavage of the proteins and the more complex protein derivatives to polypeptids of unknown complexity.

These analytical studies of the nitrogen distribution in microbe cultures furnish but little information of the identity of the products formed during the life history of the microbe, but they indicate quite definitely the quantitative character of the nitrogenous interchange between medium and microbe as growth proceeds. The metabolism of the human body is studied in precisely the same manner, and with similar methods.

It is not without significance that the diphtheria bacillus, growing either in the human body or in the glass test tube, produces the same toxin. The nitrogenous tissues of the body and the nitrogenous constituents of the culture medium alike yield the same by-product—diphtheria toxin—when they are used for energy by the diphtheria bacillus. On the other hand, the bacteria that produce their harmful effects only when they develop within the tissues of the body, as typhoid, streptococci and others, present a problem that is still obscure. The elucidation of the nature of the products of growth formed by pathogenic bacteria is one of the rich prizes this field holds forth for the future.

Notwithstanding the indefiniteness of current information regarding the chemical composition of the products formed during nitrogenous metabolism by bacteria within the body and in culture media, the general principle of the sparing action of utilizable carbohydrate for protein has been revealed by these quantitative metabolic studies, and bacterial metabolism has been brought into accord with the general phenomena of metabolism in the animal body.

PART IV. CERTAIN APPLICATIONS OF BACTERIAL METABOLISM. In a preceding section attention was directed to the dissimilarity of products formed from the common ingredients of ordinary, sugar-free cultural media by various parasitic and pathogenic bacteria. In other words, each microbe exhibits a marked specificity of chemical action, judging from the nature of the substances formed when each utilizes protein or protein derivatives for energy. Conversely, each of these respective microbes forms acidic products, chiefly or commonly lactic acid, when it utilizes carbohydrate for its energy requirements.

Not all carbohydrate configurations are utilizable for bacterial energy. Indeed, a very striking specificity exists between the protoplasmic asymmetry of bacteria and their respective abilities to utilize particular sugar configurations. The tissues of the human body are equally exacting in this respect.

This important relationship between protoplasmic asymmetry and the stereo-configuration of utilizable carbohydrate and carbohydrate-like substances has long been a theme for investigation. Pasteur (10) showed many years ago that a mold, *Penicillium*, would ferment the dextro component of ammonium racemate, leaving the laevo isomer practically intact, and this method of separating isomers by the action of biological agents was rapidly extended (11). Fischer and Thierfelder (12) examined the relations between the stereo-configurations

of certain sugars and their fermentability by yeast. From this study, and subsequent investigations upon a more extensive series of sugars and glucosides, Fischer drew his famous simile that a carbohydrate, to be acted upon, must possess a stereo-configuration that is related to the asymmetry of the cellular protoplasm as a "key is to its particular lock" (13). Subsequent studies have thrown additional light upon the interrelations of bacteria and various carbohydrates. Out of these interrelations procedures have been devised which permit of the microbial identification and estimation of various sugars and sugar derivatives (14). The correctness of the underlying principle, that "utilizable carbohydrate spares protein from utilization for energy" (15), seems to be adequately established by the very operation of this use of bacteria in carbohydrate analysis.

Many problems of interest and importance present themselves as the direct outcome of the mutual interdependence of microbial nutrition and potential microbial action. Not the least interesting and important of these is the multitude of phenomena having their origin in the alimentary canal of man. Volumes have been written upon this subject. At most, a most superficial outline can be presented here.

From birth to normal adult life the kaleidoscopic changes in the flora of the intestinal tract have their origin in the general alimentary environment (16). The food of the host is a very important factor, both in controlling the kinds of bacteria that multiply in the intestinal canal, and in determining the nature of the products these bacteria form while they are resident therein. Thus, the monotonous breast milk diet of the nursing with its preponderance of lactose creates conditions that favor the growth of the anaerobic, obligately fermentative organism, *Bacillus bifidus* (17). This microbe is devoid of more than minimal proteolytic powers; indeed, it thrives in the alimentary tract only when available lactose is continuously present. As the child grows and its dietary requirements exceed the maternal ability to provide, a noteworthy change is discernible in the intestinal flora, coincident with the altered nutritional conditions. The dietary change is essentially a marked increase in the proportion of protein to carbohydrate, which usually results in a differential enrichment of the protein residuum in the lower portion of the child's intestinal tract, with periods of carbohydrate presence and absence. Under these conditions, *Bacillus bifidus* tends to disappear, being unable to thrive in the periodic absence of carbohydrate, and more versatile bacteria take its place. *Bacillus coli* is the most conspicuous of these. It can grow well upon

the partly digested protein residua, and utilize the protein for energy during those periods when sugar is absent. That is to say, *Bacillus coli*, unlike *Bacillus bifidus*, is facultative with reference to its energy requirements (18). It is worthy of note that considerable amounts of indol are formed by it when it utilizes protein for energy, whereas it produces lactic, and smaller amounts of other acidic products when utilizable carbohydrate is available. The indol, absorbed from the alimentary tract, oxidized and paired in the liver, is a principal source of urinary indican.

This change from an obligately or nearly obligately fermentative nursing flora to a facultative putrefactive flora persists from post nursing life through adolescence to adult life. Inasmuch as experimental animals may be made to exhibit the major features of this bacterial change in type and chemical activity through careful dietary alterations (16), (17), (19), it would appear not only that diet is one important factor in determining the nature of the intestinal flora, but also that suitable dietary procedures offer a rather direct method of influencing the nature of the products formed by bacteria already present in the alimentary tract.

It is quite obvious that undesirable intestinal microbial activity may be of at least two types—one in which harmful protein derivatives are being formed, as for example in bacillary dysentery or in an overgrowth of bacteria which produce intolerable amounts of indol, or other substances that may act as slowly cumulative systemic poisons. The other type is characterized either by irritant acidic substances, as butyric acid in considerable amounts, or to unusual amounts of lactic or acetic acids.

Two fairly distinct procedures have been advocated to influence unfavorable bacterial activity in the alimentary canal. One of these consists essentially in forcing dietary procedures inimical to the growth of the microbe it is desired to supplant; the other contemplates the deliberate introduction of bacteria whose activities are of necessity favorable to the well-being of the host.

Feeding diets rich in lactose have been found helpful in infections with dysentery bacilli (20) and in typhoid fever (21). Here the primary object is to furnish the invading bacteria, and intestinal bacteria as well, with a continuous supply of carbohydrate, in order to shift the metabolism from the protein to the carbohydrate, and thus change the bacterial products formed in so far as possible to the lactic producing phase. The results of this method of therapy are quite encouraging.

In a similar manner, Dragstedt (22) has been able to produce and control parathyroid tetany in experimental animals in a very suggestive manner; upon a protein diet, the animals manifest the typical signs and symptoms of this disease; the substitution of a diet rich in carbohydrate ameliorates the symptoms very markedly.

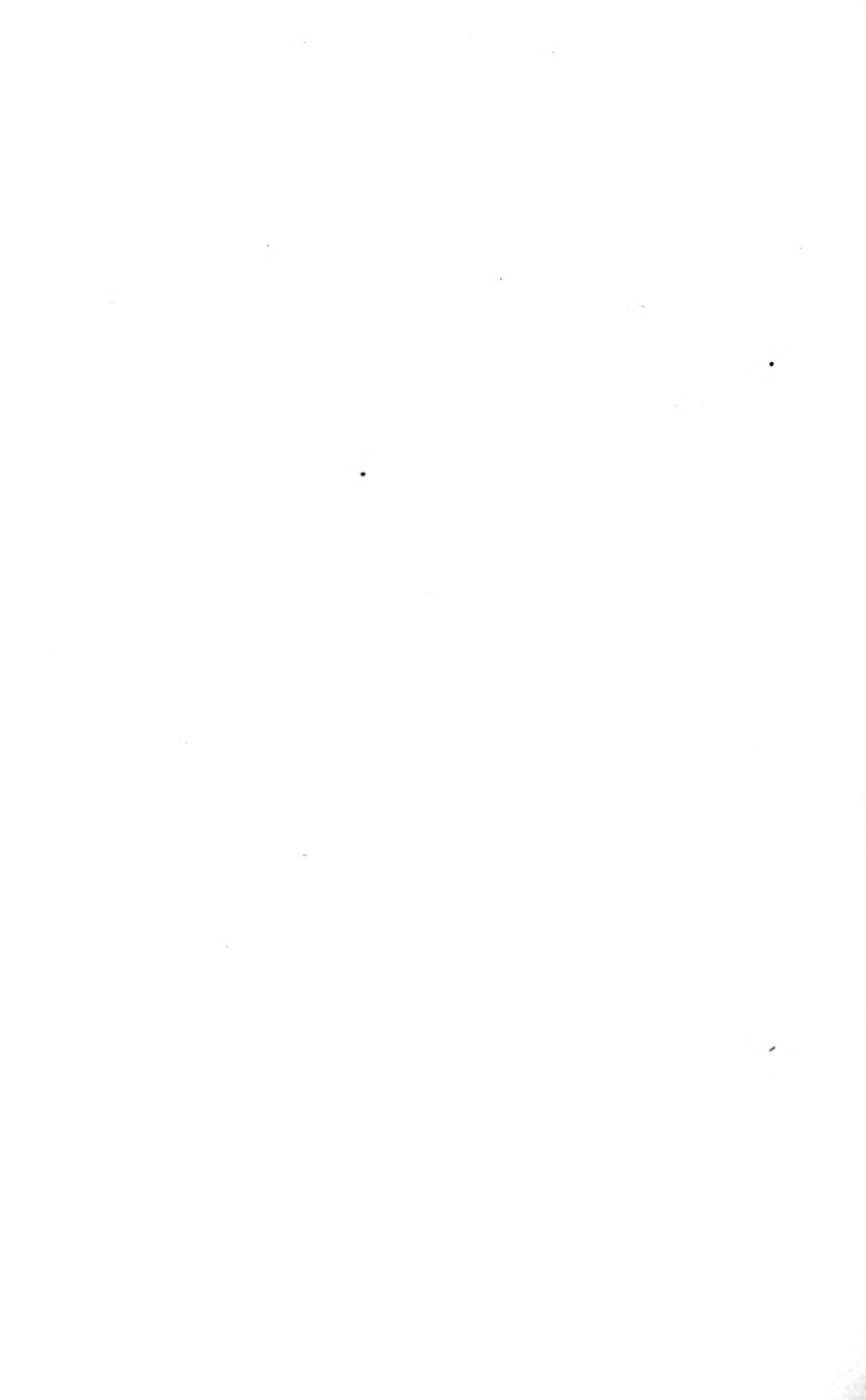
The second method—that of deliberate intestinal implantation of suitable bacteria—is as yet not well worked out but available evidence indicates that *Bacillus bifidus*, or *Bacillus acidophilus* (23), freshly isolated from the intestinal tract, so that the ability to grow in the intestinal environment is not lost, may be fed in the living state, and with suitable accessory dietary conditions may be induced to grow in the intestines. It is clear from what has been said that the implantation and growth of *bifidus* or *acidophilus* in the alimentary canal takes place only under conditions that will afford optimum conditions for the normal resident bacteria to change their metabolism to the lactic acid phase. Hence, growth and persistence of the artificially implanted bacteria is rather an index of the persistence of carbohydrate throughout the alimentary tract, with its effect upon the nature of their products of metabolism, than merely a successful inoculation with the foreign microbe. In no case is it possible to replace all the normal intestinal bacteria with *bifidus* and *acidophilus* cultures, except in very young children and animals.

The study of the chemistry of bacterial metabolism—and this is equally true for cellular metabolism in general—is in its formative state. Advances along highly important lines of the identification of individual growth products must await the development of new chemical methods. The ultimate results will carry biological science far along the path toward a real understanding of the dynamics of life itself.

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# PHYSIOLOGICAL REVIEWS

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## OESTRUS, OVULATION AND MENSTRUATION

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A long series of traditional and scientific writings testifies to the mystification produced by the menstrual periodicity of the human female. We find the Mosaic lawgivers already concerned to regulate the hygiene of menstruation; thousands of years later an Arrhenius is still puzzling over the relation between the cycles of menstruation and those of the moon. In recent years, however, there seems to have been a genuine advance toward a biological explanation of the reproductive cycle in mammals, and we may hope that the general enlightenment is finally extending to the undoubtedly peculiar problems of our own species. We now know that the discharge of ova from the ovarian follicles is a periodic function, accompanied by alterations in the female organism which provide for protection and nutrition of the embryos in their wandering and development; but the effort to test and amplify this general idea and to give it practical application in the understanding of such special cases as the human menstrual cycle has continually been hampered by a diversity of detail so great that each species seems to present a special problem. Outside the mammalian class we find such extremes as the seventeen-year cycle of the locust, *Cicada septendecim*, and the daily ovulation of the domestic hen; among the mammals we have to reckon with such different phenomena as the spectacular annual rutting of certain deer; the "heat" of horse, cow and pig, recurring perennially at three-weekly intervals; the almost totally inconspicuous oestrus of the laboratory rodents; and the menstrual hemorrhagic process of the higher primates. The dissimilarity of external manifestations is matched by the different structure of the reproductive tract and the multiplicity of form in placentation and in the embryonic membranes. The problem which presents itself to students of these phenomena is to learn the relation of ovulation to the outward events of the cycle, to follow in detail the accompanying changes of form and function in the

reproductive tract, and ultimately, let us hope, to comprehend their physiological interrelations. It is clear that the solution of the cycle of any one species must depend in part upon the progress of knowledge of them all, and that a complicated or obscure cycle like that of the human species will be intelligible only in the light of simpler types. These questions, and the recent attempts to solve them, form the subject of our review.

I. THE REPRODUCTIVE CYCLE IN GENERAL. *Oestrus*. Turning to the simplest type of reproductive manifestations, such as is presented for instance by the ungulates and the carnivores, we observe the recurrence at regular intervals of a condition characterized by sexual excitement and by impregnation of the female if copulation takes place. Between the periods of oestrus,<sup>1</sup> sexual activity is completely in abeyance. The intervals between the recurrences of oestrus, the duration of the period of sexual excitement and the degree of its manifestation are, as we have said, different in various species. The domestic pig, for instance, undergoes a three-day period of oestrus at intervals of about three weeks throughout the year; the sheep has in the autumn or, in some breeds, in spring and autumn a sequence of oestrous periods, which may include three or four cycles unless cut short by impregnation; the dog is in heat for a week or more about twice annually. In cats and dogs the inter-oestrous interval is longer than the span of gestation, while in the pig and other animals with rapid cycles the reverse is true, and there seems to be a mechanism by which oestrus (and consequently ovulation) is prevented from occurring during pregnancy.

<sup>1</sup> "Oestrus" (heat) is the periodic condition of sexual activity characterized by willingness to mate, as contrasted to the intervals during which animals do not mate. In Heape's terminology (27) the term "prooestrus" indicates the preliminary or premonitory stage immediately preceding oestrus. The interval of quietude following oestrus is called "dioestrus" if it be only a relatively brief interlude between regularly recurrent oestrous periods, "anoestrus" if it be so long as to cover a large part of the year between mating seasons. The sequent periods of prooestrus, oestrus and dioestrus or anoestrus form together the oestrous cycle. As will be seen in this review, we must also consider a post-oestrous stage; and finally, we are now aware that a stage called "metoestrus" by Heape, does not occur in the original sense except in a few animals which do not ovulate spontaneously. The reader will find in the works of Heape (27) and Marshall (45) a collection of information bearing upon this subject; the recent monograph of Long and Evans (41) contains a tabular statement of the extant data, brought up to date.

Oestrus is not in all species marked by obvious outward signs of sexual excitement. In the rodents especially it has been difficult to establish the existence of regular cycles. In the guinea pig, for example, oestrus is so inconspicuous as often to escape observation even by experienced breeders. Recently, however, it has been discovered by Stockard and Papanicolaou that the ovulation cycle is accompanied by changes in the vaginal secretion, so that by microscopic examination of vaginal smears it is possible in this species to detect a regular cycle of about sixteen days' duration. By this means we have already learned to follow the oestrous cycle of four rodent species hitherto unknown or not fully understood. (Guinea-pig, Stockard and Papanicolaou (74); rat, Long and Evans (41); mouse, Allen (1); rabbit, unpublished observations of Pelkan, quoted by Long and Evans.)

It is hardly necessary to point out the further perplexing dissimilarity between these forms of the oestrous cycle and the reproductive phenomena of the human species. Here neither sexual desire nor the act of mating are limited to special periods, but may occur at any stage of the cycle. The only distinct external event is the appearance of a menstrual hemorrhage, which in spite of its dissociation from a specific oestrus is yet so constant in its recurrence that it inevitably suggests the oestrous periodicity of other mammals.

*Ovulation.* The onset of oestrus is preceded or accompanied by changes in the ovary, particularly in the Graafian follicles and their enclosed ova. Among the large follicles in a resting stage which are always present in the sexually mature ovary, one follicle (or a group, according to the species) begins to grow still larger and passes during a few days into the fully mature stage. In the pig, for instance, the mature follicles increase from a diameter of 4 or 5 mm. to 8 or 10 mm. During this growth there is a striking development of epithelioid structure in the formerly spindle-shaped cells of the theca interna folliculi. The cumulus oöphorus is partially dissociated by thinning and spreading apart of its component granulosa cells so that the ovum is left barely anchored to the unchanged granulosa cells of the follicular wall below it. The ovum itself undergoes the phenomena of maturation, i.e., extrusion of the first polar body and the prophase and metaphase of the second polar division. The result is a distinctive modification of the follicle which is typical of the prooestrous or oestrous periods. The details of these changes in the follicle have long been known to embryologists, but their exact time-relations have only recently been made out in a few species (among them the ferret, Robinson (61); pig, Corner (10), (11);

Seekinger (68); rat, Long and Evans (41)). There has been some inexactness in the literature due to failure to recognize these morphological signs, which are essential to the identification of mature follicles.

After two centuries of discussion which is now chiefly of antiquarian interest it has been established that in most mammals the ripe follicles rupture spontaneously at some time during the oestrus, but it is also known that in three animals the follicles usually do not rupture unless coition occurs, although they undergo the preliminary maturation as in other forms; if mating does not occur, the follicles and their contained ova degenerate. This group includes the domestic cat, the ferret (*Mustelus putorius*) and the domestic rabbit. The significance and mechanism of this specialized type of follicular behavior are at present beyond conjecture. Other animals formerly supposed not to ovulate spontaneously have been removed from this class by more accurate observation.

Rupture of the follicles is affected by some unknown cause. Winiwarter and Sainmont (79) and Guttmacher and Guttmacher (22) suggest the possible action of involuntary muscle fibers in the theca externa, of which the latter authors have presented a full anatomical and pharmacological study. Other outstanding conjectures are those of Clark (8) that rupture is brought about by an increased engorgement of follicular blood vessels at the time of oestrus, and of Schochet (65) that the follicles develop in their cavities a ferment which erodes their walls.

It has been found with singular unanimity for a number of species that the unfertilized ovum passes through the Fallopian tube to degenerate in the uterine cavity, and that the passage into the uterus requires, regardless of the widely variant length of the tube in the different species, about three days' time. There are only three known exceptions to this rule. In the dog it appears that about a week's time is required for passage of the ova into the uterus. In the rat it is stated by Long and Evans (41) that the unfertilized ova do not pass farther than the last part of the Fallopian tubes; the fertilized ova require only the usual three days to reach the uterus. In the opossum (26) the ova pass more rapidly into the uterus than is the case in Eutherian mammals, reaching the uterine cavity about one day after ovulation.<sup>2</sup>

*Implantation.* The subsequent fate of the ova when fertilized has become for many species a well-grounded part of our embryological

<sup>2</sup> Sobotta (63) has given a useful review of the literature on this topic, up to 1914.

knowledge. So far as known, fertilization always takes place in the Fallopian tubes, and the fertilized ova proceed, exactly as the unfertilized, into the uterine lumen on the fourth day, by which time segmentation has begun. Thereafter (it is hardly necessary to point out) the events of further development, fixation and implantation of the early embryos differ according to the species. In some mammals, for instance the domestic pig, the embryos become fixed to the endometrium by a diffuse placental apposition so simple that there is merely a loose interlocking of fetal with maternal tissue, and no destruction of the endometrium; when at parturition the chorions are freed, they leave behind an intact maternal epithelium. In other ungulates, as the sheep and cow, there is upon the cotyledonary eminences of the endometrium a more extensive interlocking of tissue, with but little endometrial destruction. In carnivores, the embryos after fixation upon the surface of the endometrium form a placental attachment in which the endometrial epithelium and glands are ultimately destroyed, leaving only the blood vessels. In some of the rodents (i.e., the guinea pig) the ovum burrows under the endometrial surface to form a new implantation cavity, and at the same time the uterus reacts to the embryonic attack by the formation of decidual cells from the endometrial stroma. Nidation of the human embryo involves widespread destruction of the endometrium by the trophoblast, even to the opening of maternal blood vessels into intervillous spaces, and a general conversion of the endometrial stroma into decidual cells. It is not strange, in view of these differences in reaction between uterus and embryo, as found in various mammals, that the part played by the uterus in the reproductive cycle is somewhat different in various species.

*The corpus luteum.* The cavity formed by the rupture of the Graafian follicles is replaced by the transformation of the follicular walls into a new structure, the corpus luteum. The obviously important character of this tissue, or organ, has given rise to a long discussion as to its histogenesis, which is now definitely closed, or at least turned in a new direction, for we are certain that the bulk of the lutein tissue is produced by enlargement (without multiplication) of the cells of the granulosa of the follicle. Exactly what becomes of the theca interna during formation of the corpus luteum is still uncertain, or perhaps is different in various species; it has been described as becoming converted into the fibroblasts of the young corpus luteum (72) in the mouse; as degenerating in situ more or less completely, early or late in the life of the corpus luteum (marmot) (78); (man) (49); as becoming indistinguishably merged with

the granulosa lutein cells (bat) (77); and as remaining as a special cell-type intermingled with the granulosa lutein cells throughout the life of the corpus luteum (pig) (10).<sup>3</sup>

Within three to seven days after ovulation the corpus luteum has become completely organized into a body which by reason of its epithelioid type of structure, consisting of lipoid-laden cells bordered everywhere by large capillaries, has become recognized (following Born, as quoted by Fraenkel (16) and Prenant (57) as resembling the endocrine glands. This recognition, however, rests merely upon general appearances, since rather extensive cytological and microchemical studies have left us without proof of any constant or specific cellular element or secretion peculiar to this tissue. The cell-inclusions described by Regaud and Policard (59), Van der Stricht (77), Cesa-Bianchi (7), Drips (13) and others, are in the reviewer's opinion not of specific nature; similar lipoidal granulations, of equally problematical significance, occur in other endocrine tissues.

If conception follows ovulation, the corpus luteum of that ovulation persists throughout pregnancy; but if the ovum remains unfertilized, the corpus luteum (in most species) survives a much briefer time. In the pig (11), rabbit (56) and guinea pig (37), degeneration takes place after about fifteen days; in the dog (47) it is said to persist as long as one month. Although the species are few in which the duration of the corpus luteum has been worked out with accuracy, we have enough information to justify two conclusions: first, that in all known species the corpus luteum of a non-fertilized ovulation persists at least as long as the time required by the embryos of the same species to become implanted in the uterine cavity; second, with only two known exceptions (the rat and the mouse) the corpus luteum always begins to retrogress before the occurrence of a new ovulation and the consequent production of a new corpus luteum. In the case of the rat and the mouse it is true that the corpora persist so long that the ovaries ultimately contain many successive crops of undegenerate corpora lutea at one time, but even here it has been shown by an ingenious method of Long and Evans (41), (marking the living corpora with vital dyes) that there are cytological changes, presumably of retrogressive nature, which take place in each set of corpora lutea before the production of a new set.

In former years there was an extensive debate as to the existence and significance of anatomical differences between the corpus luteum of

<sup>3</sup> Readers who wish to pursue the literature of this subject further are referred to the last-cited work of the reviewer (10) for a detailed bibliography.

pregnancy (so-called "corpus luteum verum") and that of an unfertilized ovulation ("c.l. spurium"). Now that we know something of the life span of the corpus luteum, it is clear that there is but one type, which however may undergo either of two fates,—retrogression or persistence,—according to the fate of the ovum. A corpus which persists during several months of pregnancy will naturally differ in cytological detail from the younger stages of its existence. Thus the present writer (9) has been able to recognize a series of changes in the lipoid content of the lutein cells of the swine by which stages of pregnancy may be distinguished, but which (excepting the earliest changes) are not developed in the corpora lutea of unfertilized ovulation. J. W. Miller (53) has also described differences in lipoids and in calcium-content in the two varieties of the human corpus luteum. In swine, and apparently also in man, the corpora lutea continue to grow in size for a time after implantation of the embryo, and hence they reach a slightly larger diameter than those which grow but two weeks: and in swine at least they develop a heavier framework of connective tissue. It is therefore possible for an experienced observer to determine by microscopic study of a sow's corpora lutea whether or not there is a pregnancy of the second month or later, but the corpora lutea of earlier stages are not distinguishable as to pregnancy or non-pregnancy. What has just been said is confirmed by the exact work of Long and Evans (41) upon the albino rat, except that in this species, as in other rodents, the regular occurrence of an ovulation about one day after parturition gives rise to a third form of the corpus luteum, the so-called corpus luteum of lactation, which again is not fundamentally different from the others, but can be distinguished by differences in size and the dimensions of the lipoidal granules in the lutein cells.

*Function of the corpus luteum.* We may pass over the numerous older conjectures as to the function of the corpus luteum, none of which found any practical confirmation until Fraenkel (16), (17), (18) began to develop the ideas which had been given him as a death-bed legacy by his teacher, the Breslau embryologist, Gustav Born. Born had noticed that well-developed corpora lutea are found only in animals which possess a genuine placentation; in the monotremes (and as he incorrectly supposed, the marsupials) there is no corpus luteum. Furthermore, the corpus luteum of the placental mammals attains its development at the very time when the embryo is becoming attached to the uterus. Born suggested therefore that the corpus luteum is the source of an internal secretion by which the uterus is prepared for the reception and imbedding

of the ovum. At the time of Born's reflections upon this hypothesis there was only one available observation which suggested the actual existence of such a preparation of the uterus for implanting the ovum, namely, that in those animals which possess a placentation characterized by true decidua formation (man and rodents) the production of decidual cells from the uterine stroma is begun almost in the first days of pregnancy or by the beginning of the second week. It had been more or less taken for granted that the inception of the decidual changes was due to a stimulus arising from the embryo itself; but Born pointed to the occurrence of decidual changes in the uterus in tubal pregnancy and to other facts which seemed to him to indicate the dependence of the decidual preparation upon some outside influence, supposedly the corpus luteum.

Fraenkel, in planning an experimental test of Born's hypothesis, chose to use the rabbit, since at that time the early embryology of this species had been more closely followed than that of any other mammal. It was clearly known that attachment of the embryo occurs about seven days after ovulation, and that the placentation is of a decidual type. There was no further information about anatomical changes in the rabbit's uterus, serving to facilitate implantation; but assuming such a mechanism, then according to Born's hypothesis removal of the ovaries or of the corpora lutea alone during the critical six days between ovulation and implantation should prevent the attachment of the embryos. In some of Fraenkel's experiments both ovaries were ablated, with control by removal of one ovary alone; in others he cauterized the corpora lutea alone, controlling this procedure by similar trauma to other parts of the ovary without damage to the corpora lutea. The results uniformly confirmed the dependence of implantation upon integrity of the corpora lutea, for all the control animals of a large series became pregnant and all those from which the corpora lutea were totally extirpated failed to become pregnant. The outcome of this brilliant experiment has never been seriously questioned, and indeed was promptly confirmed by Marshall and Jolly (48) in a few experiments on dogs and rats, and by Niskoubina (56) in rabbits.

An equally important experimental step was taken in 1907 by Leo Loeb (36), when he conceived the idea that the maternal portion of the placenta (i.e., the decidual tissue) may be produced by the stimulus of the ova acting upon an endometrium sensitized for pregnancy by the action of the corpus luteum. Using the guinea pig for his experiments, Loeb exposed the uterus on the seventh day following an unfertilized ovulation (at which time the embryos, had they been present, would have

been about to attach themselves) and placed within the uterine cavity a small foreign body such as a piece of glass. Within four days the site of such a trauma was marked by growth of a tumor of the endometrium composed of decidual tissue. In its simplest form this beautiful experiment could be performed by simply scratching the endometrium at the proper time of the cycle. Loeb found that he could not produce deciduomata at other times of the cycle than the first week after ovulation, and that removal of the ovaries or cauterization of the young corpora lutea also prevented the development of the artificial deciduomata. Loeb's experiment has been confirmed for the albino rat by Frank (19), by Corner and Warren (12) and by Long and Evans (41). The last named writers have given a full analysis of the physiological conditions necessary for successful performance of the experiment in the rat. Gasbarrini (20), Biedl, Peters and Hofstätter (5) and Hammond (24) have also found no difficulty in repeating it in the rabbit. Kraintz (32) undertook the same experiment in dogs, in which the placenta does not contain decidual cells, and in one case succeeded to the extent that he produced by traumatizing the endometrium at the proper time of the cycle not a decidual tumor, but a simpler alteration of the endometrium closely similar to that which occurs in the placentation of this species. Passing on to mammals such as the ungulates, which have a still simpler placentation, with little or no maternal contribution to the placenta, we should expect no decidua formation nor any other reaction of the endometrium as a result of Loeb's experiment, but so far no test has been made in these animals.

*The uterine cycle.* Just after publication of Loeb's work a third fundamental addition to our ideas about the reproductive cycle was contributed from the Vienna gynecological clinic by Hitschmann and Adler (29) in a study of the histology of the human uterus. The Viennese workers were able to clear up a confusingly divergent mass of histological data by the simple procedure of studying specimens not chosen at random but classified by stages of the menstrual cycle. In brief, they found the endometrium in constant change throughout the cycle, and they described especially a period occupying a week or more previous to the onset of the menstrual flow, during which there is increasing hypertrophy and complexity of the glands, engorgement of the stroma, and conversion of the connective tissue into cells resembling the early decidual elements. These changes at their height resemble very closely the earliest decidual changes of pregnancy. Without reference to events in other mammalia, and lacking of course all knowledge as to the relation of ovulation to the

menstrual cycle in man, Hitschmann and Adler proposed as their hypothesis that the premenstrual changes represent a preparation for the reception of an ovum discharged and fertilized at some unspecified previous time in the cycle, and that actual implantation takes place in the late premenstrual stage. This work was done as a study in pathology rather than as a contribution to physiology; and although it has been repeatedly confirmed, it did not at once influence workers in the general physiology of reproduction. Indeed, what little information was then extant as to changes in the uteri of other mammalia in connection with the reproductive cycle and with the actually known progress of the ova in these species, was not in accord with Hitschmann and Adler's hypothesis. The evidence even seemed to indicate that menstruation precedes the time of ovulation. Marshall (43), (44), studying the sheep and ferret (*Mustelus putorius*), and Marshall and Jolly (47) studying the dog, had seen alterations in the endometrium during the prooestrous period, characterized by degeneration and even desquamation of the epithelium and by engorgement of the blood vessels, which in the dog went on to form hemorrhages in the subepithelial stroma and even to cause hemorrhagic discharge from the vagina; ovulation did not occur until these events were finished. Influenced by Heape's theory that menstruation in the human is homologous with prooestrus in other mammals, Marshall and Jolly considered that the degenerative changes described by them were similar to menstruation in the human, and therefore that ovulation in the human must follow menstruation; and assumed in this paper that the carnivore and ungulate uteri pass after ovulation into an inactive state, the "metoestrus" of Heape. Keller (30), however, studying in 1909 the dog's uterus anew, found after the prooestrous changes (in which he saw much less degeneration than indicated by Marshall and Jolly) a further series of postoestrous changes including hypertrophy of the glands, increased complexity of the surface foldings of the endometrium, engorgement of the stroma and hypertrophy of the stroma cells; he believed these changes to be in general similar to those seen in the human uterus by Hitschmann and Adler, and he felt that the parallelism between the species bore out the functional conception which they had imagined.

In 1910 Ancel and Bouin (2), apparently at the time unaware of Hitschmann and Adler's and of Keller's studies, began the work of placing a morphological foundation under Fraenkel's and Loeb's results by demonstrating the occurrence of actual changes in the rodent uterus concomitantly with the development of the corpora lutea. It will be

remembered that in the rabbit there is no spontaneous ovulation, and hence no formation of corpora lutea, except after coitus. Ancel and Bouin permitted rabbits to mate with males sterilized by vasectomy, and thus secured the development of corpora lutea without pregnancy. Under these conditions the uterus was found to become enlarged and hyperemic; its epithelium, both superficial and glandular, underwent mitotic proliferation, and the glands increased their complexity of ramification until there was produced a considerable alteration of texture which Ancel and Bouin interpreted as indicating a preparation for implantation. These changes were found to reach their maximum about the eighth day after ovulation, and were followed by retrogressive changes from about the tenth day onward, until at the twenty-fifth day the uterus had returned to the resting condition.

Hill and O'Donoghue (28) studied the reproductive cycle of a marsupial, *Dasyurus viverrinus*, in which there occurs once each year a period of oestrus followed by a spontaneous ovulation. They found a series of changes in the uterus, beginning even before oestrus and continuing after ovulation, characterized by hypertrophy and hyperemia of the endometrium, with increase of its vascularity and proliferation of the superficial and glandular epithelium. Since these changes occurred whether or not the animal became pregnant, Hill and O'Donoghue were led to consider them a mechanism for preparing the uterus for implantation, following upon an ovulation and formation of the corpora lutea; they applied the name of "pseudopregnancy" to the state brought about by the action of this mechanism in the absence of fertilization.<sup>4</sup>

About the same time Leo Loeb (38) described the uterine cycle of the guinea pig, which he had previously found to ovulate spontaneously every fifteen to nineteen days. He found that in this animal oestrus is accompanied by a migration of leucocytes from the uterine blood vessels through the epithelium, with the occurrence of vacuolar degeneration in the epithelial cells. Following this there is a wave of mitosis in the superficial and glandular epithelium and in the stroma, which reaches its height and ceases about the sixth day, or just before the time at which Loeb had previously found the endometrium sensitized for the production of deciduomata. These changes are identical with those of the first week of pregnancy. If the young corpora lutea are excised, the endometrial changes are inhibited until a new ovulation takes place. The cycle of the guinea pig is therefore an alternation of

<sup>4</sup> It seems preferable to avoid the suggestion of falsity or imitation inherent in the prefix *pseudo*, by using the terms *progravid*, *progestational*.

uterine activity and retrogression, following the cycle of the corpora lutea.

In 1917 Marshall and Halnan (46) took up the events of the dog's cycle once more and followed it into the postoestrous stage, confirming and extending the description, given by Keller (30), of endometrial growth changes following ovulation; they showed moreover that the development and retrogression of these changes are correlated with the cycle of the corpus luteum. In this paper Marshall dropped his former theory of a homology between prooestrus and menstruation in favor of a view which assumes that in some way menstruation represents both a prooestrus and a postoestrous degeneration condensed into one.

The same year saw the publication of an important account of the guinea pig's cycle by Stockard and Papanicolaou (74), who called attention to the occurrence, at the time of ovulation, of a series of histological alterations in the epithelium of the reproductive tract, especially in the uterus and vagina. In the vagina there is first a desquamation of large quantities of epithelial cells, and then a diapedesis of leucocytes into the lumen, so that by the observation of smears made from the vaginal secretion it is readily possible to follow the cycle and to determine the day of ovulation. In the uterus there are at this time vacuolar degenerations of some of the epithelial cells, going on even to desquamation of cells or patches of epithelium; leucocytes migrate from the endometrial vessels and there may be small hemorrhages into the lumen. We are still uncertain as to the physiological significance of these changes in the vagina and endometrium. Stockard and Papanicolaou thought them to resemble human menstruation; but whether or not this interpretation is correct, the vaginal changes serve to give us for the first time a means of following the individual cycles of living rodents, and hence are already opening up in American laboratories a promising field of research. The recent studies of Evans and Bishop (15) on the relationship of diet to the ovulation cycle indicate the possibilities afforded by Stockard and Papanicolaou's discovery.

Since very little is known about the actual histology of the uterus during the first days of pregnancy in any species, the histological workers have in general merely assumed that the postoestrous changes which they have observed are actually in the nature of adaptations for implantation. The reviewer (11) undertook to test this point for one species at least, choosing the domestic pig because of its very simple placentation. The work involved following in exact detail the ovarian cycle, the progress of the ova and early embryos, and the histological changes of the uterus

during the cycle and in early pregnancy. The results were so clear-cut as to serve almost as a diagram of the mammalian reproductive cycle as we are now beginning to understand it. In this species oestrus occurs at intervals of twenty-one days. Prooestrous enlargement of the follicles is accompanied by changes in the endometrium practically similar to those described by Stockard and Papanicolaou; during the development of the follicles and then of the corpora lutea there is a wave of cell division in the endometrium; then as the early embryos are being transported and spaced within the uterus the epithelial cells assume a high columnar arrangement, as if entering a secretory phase. Next there is a stage in which the epithelium is roughened upon its surface by the extrusion of cytoplasmic processes; this stage lasts exactly as long as the period required for the actual attachment of the chorions. Up to this point (the fifteenth day) the process is the same whether or not the sow has been impregnated, but if she is not pregnant the corpus luteum suddenly retrogresses and with it the uterine epithelium returns without obvious destruction to the prooestrous stage as new follicles develop. There are parallel changes in the glands, but on the whole the alterations are mostly in the epithelium, probably because the pig has a diffuse superficial placentation; in mammals with more complex placentation the stroma and glands of the uterus are called upon for a greater share in the pronidational changes. An almost exactly similar cycle in the cow has since been described by Zietschmann (81), although without data as to the progress of the ova and embryos.

The studies of Long and Evans on the albino rat (monograph (41) in 1922 after numerous preliminary studies) are of extraordinary fulness and interest. By application of Stockard's methods they have determined the rat's cycle to be of but four to six days' duration. During this time there is a periodic alternation of histological changes in the uterus and vagina, following the maturation of follicles and the development of corpora lutea; but the frequent renewal of ovulation seems to cut short the full development of these changes. However, if a copulation occurs, the next ovulation is inhibited, and the corpora lutea persist without interruption; in this case the genital tract undergoes the alterations of pregnancy. If copulation be initiated by the insertion of a glass rod into the cervix uteri at the time of oestrus, then the same inhibition of ovulation occurs, and the genital tract undergoes alterations resembling those seen in pregnancy. This statement is based chiefly on Long and Evans' description of the vaginal cycle; they have not yet followed the uterine changes in the period following artificial prolongation of the cycle,

but they have found that it is only in such a period that artificial deciduomata can be regularly produced by Loeb's experiment. The rat's cycle as worked out by these authors represents therefore a highly specialized speeding up of the ovulation rate, with the development of a peculiar mechanism by which, in the event of mating, time is nevertheless provided for the development of the same series of post-oestrous physiological reactions which is seen in other mammalia.

*Other mechanisms of the cycle.* Recent investigations have begun to reveal the existence of other mechanisms at play in the complicated process of reproduction. There is a cycle of histological change in the Fallopian tube, long suspected, but not fully studied in systematically dated material. Snyder (71) has recently followed the cycle of the pig's Fallopian tube, and has described a sequence of changes in the epithelium parallel with the ovarian cycle. Observations of Moreaux (55) and others on the rabbit's tube indicate the occurrence there of still more elaborate processes of secretory nature, probably concerned with producing the mucoid coating with which the ovum is provided in this species. Another important field seems to be opening out of the work of Blair (6), Keye (31) and Seekinger (68), who have discovered cyclic variations in the spontaneous activity of the uterine and tubal musculature. As Keye and Seekinger have traced them in the pig, these variations follow with impressive regularity the cycle of the ovary. A strip of the tubal or uterine wall, suspended in oxygenated Locke's solution, continues to undergo regular contractions *in vitro*. Since these contractions change in rate and amplitude throughout the cycle, the resultant curve is characteristic for each stage. For instance, a particular type of contraction wave, characterized by an undulating alternation of large and small excursions, is obtained from the tube when the ova are in passage through the tubal canal and from the uterus a few days later when the degenerating ova or early embryos are in the uterine cavity. The significance of this contraction cycle can only be conjectured; Keye and Seekinger suggest the possibility that it represents a mechanism for transportation of the ova and spacing of the embryos in the uterus. The reviewer, with Seekinger, has in preparation the description of an exactly similar cycle in the tube of the rhesus monkey.

Through the studies of Ancel and Bouin (3) it is known that there are proliferative changes in the mammary gland parallel with the development of the corpus luteum. This work done on the rabbit has been confirmed by Hammond and Marshall (25). In species with spontaneous ovulation, and hence with periodic new formation of corpora lutea, it

appears that the mammary gland participates in each cycle, by vascular congestion and proliferation of its acini (39), (40). No doubt a similar process is responsible for the premenstrual mammary discomfort, common in women.

In spite of their many gaps and differences the contributions which we have reviewed have led us to a clear physiological conception of the reproductive cycle. Diagrammatically expressed, the growth of the follicle is accompanied by sexual activity and by prooestrous changes in the genital tract; after ovulation the corpus luteum brings about the progestational alterations in the tube, uterus and mammary glands. Omission of pregnancy and consequent early retrogression of the corpus luteum is followed in some animals by a long resting stage (anoestrus), in others by a brief interval (dioestrus), in still others by premature onset of a new ovarian cycle. In most animals these mechanisms occur in full at each ovulation; in others, as the rat and rabbit, there are special modifications by which the events of the progestational period are cut short. This conception, pieced together from many species by various means, is at the moment passing from the status of hypothesis to that of demonstrated fact; but much remains obscure. The post-oestrous modifications are perhaps most clearly comprehensible and subject to correlation; at least the differences between species are theoretically explainable by known differences in placentation. A greater problem is presented by the prooestrous changes of the uterus. It happens that those species about which several observers agree in the description of vacuolar degenerations, leucocytosis and mitosis in the epithelium at this stage, are all animals with rapid cycles, in which new follicles ripen at once after degeneration of the corpora lutea; thus it is not clear which of the prooestrous changes are correlated with the retrogressing corpus luteum and which with the new follicle and oestrus. The importance of this matter is obvious in connection with menstruation.

Finally, it will be seen that our information is chiefly in the nature of a chronological correlation between the outward events of the cycle, the ovarian and uterine changes, and the movement and implantation of the ova. The actual causal relationships, endocrinal or otherwise, between the follicle and corpus luteum on one hand and the uterus on the other, are as yet largely unexplored.

II. THE HUMAN CYCLE. In the human cycle there is no sharply defined oestral period to serve as a clue to the moment of ovulation, and moreover the occurrence of menstruation adds a complicating factor

present only in the higher primates. These differences, and the difficulties which hamper study of reproduction in our own species, have left us up to the present with a very incomplete knowledge of the menstrual cycle. Menstruation itself is variously thought (*a*) to be an independent or interpolated event with no relation to ovulation; (*b*) to represent oestrus; (*c*) to be a prooestrous event; (*d*) to be the retrogressive stage of the human cycle, after preparation of the endometrium for an ovum which does not become implanted. The heart of the difficulty obviously lies in our ignorance of the time-relation of ovulation and implantation with respect to menstruation. We do not know, by any direct observation, when the primate ovum is discharged; the unfertilized ovum has never been observed after leaving the ovary, nor has the fertilized egg been seen early enough to give us any information in regard to the earliest stages of implantation, i.e., during the first two weeks. Numerous efforts have been made to estimate the rate of ovulation with reference to menstruation from human embryos of the third week and later. The age of the embryo is estimated by one means or another, and the date of ovulation is calculated backward from this. Such conjectures have engaged some of the most eminent embryologists (His, Mall, Teacher and Bryce, Grosser, Von Spee, Triepel)<sup>5</sup> but the results have variously placed ovulation at any and every stage of the cycle. The method is essentially a begging of the question, and is furthermore dependent upon scanty material and takes too little account of embryonic retardation and abnormality.

It is sometimes possible to obtain in connection with a human embryo or child an exact record of the copulation date. The date of ovulation calculated from such a record would of course in the other mammalia be the same, within a day or two, as the copulation date, since these animals mate only near the time of ovulation; but in man the calculation of embryonic age from a single copulation is far from certain and indeed is often in disagreement with other data or the probabilities of the case. The great war gave to German obstetricians a singular opportunity to study the relation of conception to the menstrual cycle. Many pregnancies of soldiers' wives dated from brief furloughs of their husbands from the front. Some thousands of such cases were collected by Siegel (70) and Pryll (58) and others (see also Zangemeister (80)), and when assembled gave curves showing that conception may result from mating at any stage of the cycle, except that the last six or seven days preceding menstruation are practically sterile; fertility is highest about one week

<sup>5</sup> For literature of this subject see Mall (42) and Triepel (75)

after menstruation and falls at first slowly and then rapidly as the last week of the intermenstrual period approaches. To explain such a result we must needs assume that something is different from the state of affairs in other mammals; either ovulation may occur at any time of the menstrual cycle, or else the germ cells, spermatozoa or ova, or both, are able to survive for a week or two in the female reproductive tract and then take part in zygosis.

More light has been won by direct study of the ovaries. A mass of earlier work, which we shall pass by, was based on the ancient hypothesis, still widely current among the laity, that menstruation is homologous with oestrus; but among the medical profession this notion has largely given place to the view of Leopold and his colleagues (33), (34), (35), who gathered together a large number of human ovaries from operations and attempted to correlate the appearance of the corpus luteum with the menstrual history. These workers made their determinations by macroscopic observation, in accordance with a more or less preconceived idea of the corpus luteum as it was supposed to appear at each of the four weeks of the cycle; at the present time, although we are still largely ignorant as to the rate of organization of the human corpus luteum, we know enough of the facts as they exist in other animals to throw great doubt upon the value of Leopold's criteria for determination of the age of corpora lutea.

Leopold and his colleagues believed that they found corpora lutea of various ages at each stage of the menstrual cycle, and therefore they taught that there is no close correlation between ovulation and menstruation; the two events may coincide or may differ widely in point of time. This teaching, although sterile as regards physiological interpretation of the human cycle, has found numerous supporters, the most recent being Schickelé (64); but it should be noted that some of the authors have failed to distinguish between the two ideas (*a*) that there is no correlation between ovulation and menstruation, and (*b*) that the two events are merely not coincident. There is a tendency to assume the first of these statements upon proof of the latter alone.

In 1902 Fraenkel (16), (17), (18) began the exposition of his conception of the corpus luteum cycle. We have already recounted in the preceding section that part of Fraenkel's work which dealt with the theory and the proof that the corpus luteum is an organ of internal secretion by which the uterus is prepared for implantation of fertilized ova. This general idea was accompanied by a special theory of the human cycle, which Fraenkel briefly stated as follows: a follicle grows, ruptures and

is converted into a corpus luteum; the ovum is discharged and transported through the tube; meanwhile the uterus undergoes changes preparatory to nidation of the embryo, by which it attains the premenstrual type of structure. The premenstrual stage of the human is thus akin to the post-oestrous stage of other mammals. If fertilization and implantation do not occur, the endometrium breaks down and menstruation is the result. This hypothesis, foreshadowed by the older conjectures of Aveling, Sigismund and Beard, had the initial merit of proposing a definite physiological idea subject to precise investigation. It was to find almost immediate support in the histological observations of Hitschmann and Adler which we have already quoted; meanwhile Fraenkel himself undertook to test it by renewed observations of the ovary after the method used by Leopold and others. However, his experimental work on the rabbit had taught him the dangers of macroscopic examination of the corpus luteum, and he therefore made no attempt to diagnose other than "recent" corpora lutea with fresh red tissue at the points of follicular rupture. Moreover, to avoid pathological cases he made his studies during life by direct observation of the undisturbed ovaries of women subjected to laparotomy for conditions other than those of a grave gynecological nature. He found recent corpora lutea only during the second half of the interval, and was even willing to name as the average date of ovulation the nineteenth day after the onset of four-weekly menstruation, or the ninth day before the onset of the next period. The corpus luteum he believed to reach its full development about eight days later, to begin retrogression at the onset of menstruation, and to disappear at the end of this period. These observations were confirmed by Ancel and Villemin (4), who placed ovulation however twelve days before menstruation, as well as by various pupils of Fraenkel, the latest of whom is Tschirdewahn (76). Fraenkel also attempted a physiological test of his theory by cauterization of the corpus luteum in women subjected to laparotomy for various surgical reasons. In his hands this procedure resulted in postponement of the next menstruation for three to eight weeks. Halban and Kohler (23) and others have, on the contrary, found it to bring on menstruation within a few days. Such experiments and the discussion which they have aroused regarding the exact causal relation of the corpus luteum to menstruation will hardly afford exact conclusions until we are in a position to extirpate the corpus at much more precisely dated stages.

Another group of workers, realizing the uncertainties of bare macroscopic study of the ovary, have returned to the examination of excised

organs from the operating room, attempting to apply both the histological criteria of the corpus luteum recently gained from other species, and the newer knowledge of the uterine cycle as introduced by Hitschmann and Adler. The material available for such study contains almost no specimens which would be accepted by workers dealing with laboratory mammals, but after discarding the graver lesions and the more inaccurate histories there remain a few specimens comprising both ovaries, the endometrium, and a passable menstrual anamensis. Robert Meyer and Carl Ruge II (52) were able to assemble 106 specimens, of which a portion met the above requirement. Interpreting the ovaries in accordance with R. Meyer's standardization (49) of the human corpus luteum into stages of proliferation, vascularization, maturity and retrogression, they found that there is a fairly high correlation between the stages of the corpus luteum and the stage of the endometrium. The recent corpus luteum (stage of vascularization) accompanies the early premenstrual endometrium, the mature corpus luteum accompanies the later premenstrual endometrium, and retrogression of the corpus luteum generally begins about the onset of menstruation. In their first papers Meyer and Ruge placed ovulation in the first half of the intermenstrual interval, most commonly during the first postmenstrual week, but later Ruge (62), (63) modified his view by dating ovulation between the eighth and fourteenth day after onset of the menstrual flow. Meyer and Ruge's correlation between the stages of the corpus luteum and the uterus is in general convincing, based as it is upon objective comparisons between histological preparations and in no way dependent upon individual histories; and Ruge's estimate of the date of ovulation seems, like Fraenkel's, at least an approach to the truth. It must be remembered, however, that for want of actual observation of the human ovum we are still without a starting point for determination of the maturity of follicles and the age of corpora lutea. Any dating based upon a classification of stages of the corpus luteum, such as Robert Meyer's, involves an assumed comparison with other forms, and hence adds another possible source of error to those inherent in human material from the clinic, with all its variation in age and pathological condition. The effort to calculate backward to the date of ovulation from the anatomical condition of the ovaries is thus still inaccurate, though great advance has been made over Leopold's methods. No destructive criticism is intended here, however, for it is after all the especial contribution of the modern clinical workers in this field, led by Fraenkel, by Meyer and Ruge and by Schroeder, that they have utilized as far as possible the conceptions already learned from other species.

Schroeder (66),(67) has studied about 100 cases from his own clinical material, with results which appear to the present reviewer to approximate the best that can be done with such material with methods available at present to the clinical laboratory. His descriptions of the corpus luteum are in rather close accord with the experience of those who have studied other mammals under more favorable conditions, and he appears to have done much to eliminate inaccuracies due to cycles of abnormal duration and similar causes. Schroeder concludes that rupture of the follicle takes place at about the fourteenth to the sixteenth day after onset of menstrual flow, and that the corpus luteum is a solid structure within three to five days, thus reaching maturity at about the eighteenth or the twentieth day of the four-weekly cycle. The corpus luteum may persist into menstruation, but is undergoing retrogression by the end of the flow. Just as described by Meyer and Ruge, the interdependence of the corpus luteum and the endometrium is very close, the mature corpus always being found in association with a premenstrual endometrium.

The gradually increasing accuracy of such studies has suggested the possibility of more precise examination of the primate ovary along experimental lines. Reusch (60), assuming the occurrence of ovulation about the middle of the interval, took advantage of two laparotomies done upon women who were menstruating regularly, to examine the ovaries on the thirteenth and the eleventh day, respectively, preceding the calculated onset of menstruation. In each case he found and excised a corpus luteum which proved on section to be comparable with the earliest stages of the corpus luteum as found in other mammalia. Unfortunately there was no opportunity to study the endometrium.

The present reviewer has in preparation the preliminary report of an experimental attempt, now in progress, to establish the ovulation cycle of a primate, *Macacus rhesus*. This familiar monkey undergoes menstrual phenomena to all appearances quite like the human, with a cycle averaging about four weeks. During the present year it has twice been possible to discover the ovum in progress through the reproductive canal. These are the first authenticated unfertilized ova of primates yet seen after discharge from the ovary. In one case the animal was killed about the twelfth day before the onset of the next expected menstruation; the ovary contained a recently ruptured follicle, and the mature ovum was found in the corresponding Fallopian tube. The endometrium closely resembled the late interval stage of the human as classified by Hitschman and Adler. The second macaque was killed about the seventh day

before the calculated onset of menstruation; there was a large solid and mature corpus luteum, and the ovum, in a state of moderately advanced degeneration, was found in the uterine cavity. The endometrium was in an early premenstrual stage. Examination of the ovaries at other periods of the cycle showed a relation of follicles and corpora lutea to the endometrial cycle which bore out, at least in fully mature animals, the correlations of Meyer and Ruge and of Schroeder.

It will be seen that by these studies of the primate cycle, incomplete as they are, we have advanced beyond the conjectures which still occupy much of the literature, and that we cannot be far from the truth about the time of ovulation and the growth of the corpus luteum. There is less to say as yet about the other phase of the human cycle; that is, menstruation and the retrogression of the corpus luteum. The exact function of the menstrual hemorrhage is at present in doubt. The original view of Fraenkel (at least as interpreted by his earlier readers) that the corpus luteum by its activity brings on menstruation, has by almost common consent been reformulated to propose that it is the retrogression of the corpus luteum which causes breakdown and hemorrhage from the endometrium, no longer subject to anabolic stimuli from the lutein cells. If the ovum be fertilized, it is assumed that the embryo causes persistence of the corpus luteum, which in turn continues its progestational effect upon the endometrium, and thus postpones menstruation. Menstruation therefore (though by no means an abortion of the unfertilized ovum, as it is sometimes put) is on this theory merely a violent demolition of the premenstrual uterine edifice, some days after the expected tenant fails to arrive. The only normal outcome of an ovulation is pregnancy, and the hemorrhage is viewed as a pathological result of failure in this respect. I have mentioned on the other hand Schroeder's findings that the corpus luteum at least sometimes does not begin degeneration until after the onset of menstruation. One or two trustworthy human specimens in the author's small collection also show the corpus luteum still undegenerated (as far as can be revealed by the ordinary staining technique) on the first days of menstruation. It is, I believe, not beyond conjecture that the pronidational preparation of the endometrium goes actually to the verge of menstruation. We are of course ignorant as to the exact relation of human implantation to the cyclic changes of the ovary and uterus: the most valuable observation yet at hand is given by Grosser (21), in a spectacular photograph of the Kleinhans specimen, which shows a very young human embryo in the earliest stage of implantation, upon an endometrium which is typically

premenstrual. Decidual alteration of the stroma cells is just beginning, and there are a few red blood cells in the stroma, but no large extravasations. It has also been noted in connection with a number of the earlier human embryos (at stages slightly later than the Kleinhans ovum) that the endometrial glands contain blood even at a distance from the invading trophoblast (cf. Von Möllendorff (54)). In many pregnancies there is even a trace of vaginal hemorrhage at the first lapsed menstrual period. My conjecture is thus that in order to provide for the highly specialized embryonic implantation of primates, with its opening of the maternal blood vessels into the intervillous spaces, the endometrial process is carried so far as even to cause bleeding into the tissues during the last days of the interval, at the time during which the early embryo is to be implanted. The action of the embryo alters the latter part of the process so as to inhibit or limit the hemorrhage; but if no embryo be present to utilize the extravasation, then the blood escapes into the uterine lumen and externally visible bleeding occurs. Such a hypothesis seems to render the menstrual process something more than a pathological wastage, and also suggests a reason for its total absence in species which have less specialized forms of implantation: but there can be no solution of these queries until in man or some other primate we possess a sufficient series of embryos of the second week to give us facts instead of hypotheses.

Without exact knowledge we are also unable to deal fully with a doctrine which is having its latest recrudescence in the works of R. Meyer (50), (51) and others,—the theory of “primacy of the ovum,”—which assumes that it is the egg-cell itself which by its growth brings on through some hormonal effect the positive phase of the menstrual cycle and by its death when not fertilized causes destruction of the endometrium. The reviewer can add little to the objections already leveled at this hypothesis by Seitz (69) and by Evans (14), except to refer once more to his own description of a monkey in which the ovum was actually found in degeneration while the endometrium was as yet premenstrual. There is every reason to suppose that in man and the monkey as in other mammals the unfertilized ovum has entirely disintegrated long before completion of the progestational stage of the uterus. An ovum which is non-existent can surely not long affect the uterus. We have, no doubt, a long time to wait for chemical identification of the reproductive hormones and their exact sources; meanwhile the burden of proof is upon those who discount the physiological possibilities of the general ovarian apparatus, including especially the follicles and the corpus

luteum, in favor of so minute and detached an object as the mammalian egg.

In summary, it may fairly be stated that the trend of recent work is to suggest the basic similarity of the primate reproductive cycle to that of the other mammalia as outlined in the first section of this review. We have good reason to believe in the occurrence of periodic maturation and discharge of the Graafian follicles at a time near the middle of the interval (in the typical four-weekly cycle) or ten to fifteen days preceding the onset of menstruation. The corpus luteum probably reaches maturity in a few days, as in other species, and persists throughout the last week of the interval, to begin its retrogression about the time of menstruation. If the ovum is not fertilized, it degenerates in the uterus a few days after its discharge (a conjecture for which we have as evidence, as far as primate species are concerned, only the one monkey's ovum mentioned above); but if fertilized, it reaches the uterus during the stage of premenstrual alteration of the endometrium which accompanies the mature corpus luteum. At this time we may suppose the uterine stroma is sensitized for the production of decidual cells and is ready to receive the embryo. If fertilization and imbedding do not occur, the premenstrual endometrium proceeds to the stage of menstrual hemorrhage.

In adopting for the purposes of further study so satisfactory an explanation of the primate cycle, we must admit that it does not meet, without additional conjecture, a difficulty presented by the accepted belief that the human female is fertile at all times of the cycle, except possibly during the last week of the intermenstrual period as shown by Siegel and others. This difficulty can be theoretically explained in several ways. Granting that ovulation is usually regular and narrowly limited to a particular time of the cycle, there may be either extra-cyclic ovulations, possibly induced by sexual stimulation, as suggested by Triepel (75) and Evans (14), or else the germ-cells are able to survive in the female reproductive tract for as much as a fortnight. In other mammals (so far as known) the unfertilized ova begin to degenerate by the end of their three-day sojourn in the tube. As to the possible survival of spermatozoa in the human Fallopian tube, we have conflicting reports. Observations on other mammals with limited oestrous periods are obviously irrelevant, since mating occurs only at the time of ovulation, and survival of spermatozoa would be useless. The question needs serious investigation. Meanwhile it may be said that if we accept a regular ovulation occurring about twelve days before onset of menstruation, and assume the viability of spermatozoa in the oviduct for a space

of two weeks following coitus, then all the known interrelations of the human reproductive cycle can be seen to follow. Finally there is the bare possibility that if all causes of error could be ruled out of such statistics as Siegel's the human female might actually be found to be fertile only during a limited portion of each cycle near the day of ovulation.

The whole story illustrates anew the interdependence of physiological and morphological methods. Without the classical labors of von Baer and a long succession of embryologists we should not have had even a clue to this grave question of human physiology; in a later period of the investigation we have again seen the histologist point the way, but only after he had learned to classify his material by functional stages. In the end, the problem as it especially concerns our own species will be solved by taking into the clinic the great advances of method now in the making by physiological anatomists.

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## THE FILTERABLE VIRUSES

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The term "filterable viruses" was introduced in the late nineties of the preceding century, to denote a group of disease producing agents, which seemed to differ from other forms of living matter in their ability to pass through earthenware filters having a pore diameter smaller than the smallest bacteria then known. They were discovered at a time when the inability to demonstrate or isolate the causative agents of many infectious diseases affecting man and animals had raised the question of the possible existence of ultramicroscopic organisms.

Inasmuch as none of the viruses, in the early years of their discovery, had been demonstrated with the microscope, the term ultramicroscopic organisms was used synonymously with the term filterable viruses. Following the discovery by Borrel, v. Prowazek, Lipschuetz, Volpino, Paschen and others, however, that in a number of diseases the causative filterable agent can be rendered visible by suitable methods of concentration and staining, the term ultramicroscopic organism was abandoned, even though many of the filterable viruses still remain in the domain of the invisible. Physicists have expressed the opinion that these are not ultramicroscopic, however, in the sense that their minute size renders them invisible, but that they have escaped detection for want of a suitable method of illumination. As a matter of fact no representative of the filterable viruses has yet been demonstrated with the ultramicroscope which could not also be seen with the usual high power lenses in the dark field. For practical purposes then the term filterable viruses may be retained, even though we are conscious that the group in all probability is not a homogeneous one.

The first representative of the filterable viruses was discovered in 1892 by Iwanowski (1) in the course of his investigations into the etiology of the mosaic disease of tobacco. He found that he was able to produce the disease in healthy plants by inoculating them with the filtered juice obtained from diseased leaves. He also established the remarkable viability of the filtered virus, which remained active for ten months. This characteristic is shared by many representatives

of the group, and in itself seems to differentiate them from the common disease producing bacteria and protozoa.

In 1898 Beijerinck (2) published the results of his observations on the etiology of the same disease, evidently in ignorance of Iwanowski's earlier work, arriving at similar conclusions.

In the same year there also appeared Loeffler and Froesch's (3) epoch-making studies on the etiology of hoof and mouth disease. These investigators found that they could produce the disease in question by inoculating animals with Berkefeld filtrates of the contents of the specific vesicles, and that the disease could thus be transmitted in passage ad infinitum. They fully realized the basic importance of their results, and suggested that other infectious diseases of hitherto unknown etiology, such as variola, vaccinia, scarlatina, measles, Rinderpest, etc., might be due to organisms of this order. Their predictions have been amply fulfilled, for in his collective survey of the subject, published in 1913, Lipschuetz (4) could present a list of forty-one diseases affecting man and various animals, in which the filterable nature of the causative agent had been established with more or less certainty. Of human diseases this list includes yellow fever, rabies, molluscum contagiosum, dengue, warts, pappataci fever, variola, trachoma, epidemic parotitis, poliomyelitis, scarlatina, measles, alastrim and inclusion blenorrea, while of animal diseases the following are represented: hoof and mouth disease, pleuropneumonia of cattle, South African horse sickness, chicken plague, epithelioma contagiosum, sheep pox, Rinderpest, hog cholera, catarrhal fever of sheep, heart water disease of sheep, agalaktia contagiosa, benign papulous stomatitis of cattle, leukemia of chickens, myxomatosis of rabbits, Nairobi disease of sheep, jaundice of silk worms, Rous' filterable sarcoma of chickens, distemper of horses, besides certain epidemic diseases which have been described as affecting black birds, rats and guinea pigs.

Since 1913 a number of additional diseases have been more or less definitely linked up with the activity of filterable viruses, such as the d'Herelle disease of bacteria, the mosaic disease of tomatoes, cucumbers, lettuce, beans, etc.; further the wilt disease of the gypsy moth, the sacbrood disease of bees, and amongst diseases affecting man, paravaccinia, influenza, the various types of herpes, and possibly also epidemic encephalitis.

In the present survey it is the writer's intention to discuss in some detail certain phases of the more modern work that has been done in connection with the filterable viruses, rather than to go over the achieve-

ments of earlier workers, excepting in so far as this may be necessary for the proper understanding of the subject.

THE NATURE OF THE FILTERABLE VIRUSES. 1. *The question of their corpuscular character and size.* In discussing the nature of the filterable viruses, it is necessary to bear in mind that mere filterability can hardly be regarded as sufficient grounds for assuming that the viruses represent a homogeneous group, and it is quite possible that what may be found to hold good for one or more representatives, does not hold good for others. A priori it is thus possible that some of the viruses may not be of corpuscular nature. This possibility was discussed already by Beijerinck, who expressed the belief that the virus which causes the mosaic disease of tobacco was a *contagium vivum fluidum*. He came to the conclusion that the virus was not corpuscular, but present in solution, on the basis of the following experiment. Ground up, diseased leaves were placed on thick agar plates and left for ten days. The surface was then washed off with water and a strong solution of bichloride of mercury, and the top layer of the agar removed; the bottom layers nevertheless proved to be infective. Inasmuch as Beijerinck's studies of the disease along bacteriological lines had only led to negative results, and filtration experiments with very dense porcelain filters had shown that the virus could pass through the pores, his conclusion that the infective principle was present in solution does not seem unnatural. The puzzling point, however, was that it proved possible to infect an unlimited number of leaves by starting with the minutest quantity of the virus, so that the conclusion seemed unavoidable that the virus actually multiplied in the plant. In this manner he was led to assume the existence not only of a *contagium fluidum*, but of a *contagium vivum fluidum*.

Later investigations made in connection with various other viruses have brought forth no evidence to suggest that any of them are not of corpuscular nature. Following the discovery on the part of Giemsa and v. Prowazek (5) that the virus of chicken plague can be held back by agar ultra filters, the application of the same or similar methods to the study of other viruses showed that all that were tested were held back. By making use of graded ultra filters, i.e., of ultra filters of varying density, it further proved possible, not only to demonstrate the corpuscular character of various viruses, but also to determine their size. Andriewsky (6) thus came to the conclusion that the virus of chicken plague must consist of aggregates which are smaller than those of hemoglobin, viz., smaller than 23 to 25 $\mu$ m. Duggar and Karrer (7)

have similarly shown that the infective particles which cause the mosaic disease of tobacco have approximately the same diameter as the hemoglobin molecule which Beechold calculated to be from 33 to 36 $\mu$ .

In favor of the corpuscular nature of a number of viruses are also the results which have been obtained by sedimentation and centrifugation experiments. v. Prowazek (8) could thus show, in the case of the vaccine virus, that on prolonged standing the upper layers of vaccinia filtrates became non-infective or at least less infective. This experiment was thus confirmatory of an older diffusion experiment of Chauveau's. This investigator layered vaccine lymph under water, and then found after some time that the proteins and salts had diffused into the upper layers, while none of the virus could be demonstrated here.

By centrifugalizing emulsions of rabies and chicken plague containing material, for a long time and at high speed, Remlinger and Landsteiner (9), Barrett and Russ similarly found that the virus was finally present in greater concentration in the lower than in the upper part of the tube.

Quite recently MacCallum and Oppenheimer (10) further showed that it is possible to shift the position of the vaccine virus in a mixture of glycerin and Locke's solution, by changing the specific gravity of the mixture. On examining the surface layer in one instance in which the virus had been brought to the top, these investigators were able to demonstrate the presence of innumerable tiny granules, which could be stained by Giemsa's method.

2. *Are the filterable viruses animate or inanimate?* In discussing this question it should again be emphasized that any conclusions which may be reached in reference to the animate or inanimate nature of a given virus should not be generalized, that they hold good for the special virus under consideration, and for this only.

As has been pointed out above, Beijerinck came to the conclusion that the virus causing the mosaic disease of tobacco was of non-corpuscular nature, but he nevertheless endowed it with one of the most striking properties of living matter, viz., the power of reproduction. In spite of the fact that no one since has suggested the possible existence of a *contagium vivum fluidum*, in the sense of Beijerinck, the idea, nevertheless, deserves more than passing notice. Because it is possible, by starting with an infinitesimally small quantity of the virus, to serially infect an endless number of plants, Beijerinck naturally concluded that the virus must reproduce itself. But, inasmuch as it

was impossible to produce the disease in a leaf which had already unfolded, while it readily developed in leaves which were in the stage of the Anlage at the time of the inoculation, Beijerinck inferred that the virus can reproduce itself only when it is bound to the protoplasm of the cells of the Anlage. Regarding the manner in which this occurs, he offers no suggestion, however.

The question now arises, even though the concept of a *contagium vivum fluidum* cannot be upheld, whether a *contagium inanimatum* may not exist, irrespective of its physical state. In this connection I would refer in some detail to certain observations which Bauer (11) published in 1904, and which are scarcely known to animal pathologists, notwithstanding their evident basic importance. In discussing the etiology of a form of variegation affecting certain Malvaceae, which is known to plant pathologists as infectious chlorosis, Bauer pointed out that the disease in question cannot be transmitted to healthy plants by inoculation of the expressed juice from diseased leaves, but only by grafting. After a plant has once become infected, an endless number of diseased descendants may be obtained by the simple process of planting infected twigs. As in the mosaic disease of tobacco, here also only those leaves develop the malady, which were in the stage of the Anlage at the time of grafting, or shortly thereafter. Bauer now argues as follows: The disease in question is a typical infectious disease, inasmuch as it is possible to infect an unlimited number of healthy plants with grafts of even single diseased leaves. The virus must hence multiply, but it cannot be living matter itself for the reason that the disease can be transmitted by grafting only. He suggests that the virus need not multiply in the ordinarily accepted sense of the term, however, but that it may be possible that some abnormal metabolic product of the diseased leaf may function as the virus and stimulate the formation of the same product on the part of the cells in the Anlage. As a consequence leaves would develop in which the identical defect would exist, as in those with which we started, and the continued production of the same substance would affect subsequently developing Anlagen in the same way. In other words, Bauer postulates the existence of a virus of the very type that we suggested above, viz., a *contagium inanimatum*.

Of great interest in connection with this question, further, are the developments which have occurred in the course of the study of the so-called d'Herelle phenomenon. It will be recalled that d'Herelle (12) discovered the presence in the stools of convalescent dysentery patients

of a substance which was capable of passing through the pores of porcelain filters and, when added to an emulsion of dysentery bacilli, brought about their dissolution. On transferring a loopful of the contents of this mixture to a second tube containing an emulsion of dysentery bacilli, these also dissolved. This process could be continued indefinitely, and it appeared, moreover, that during passage the activity of the lytic principle underwent an actual increase. On the basis of these observations and further studies, d'Herelle concluded that the lytic agent must be endowed with life, as it evidently multiplied in the course of its transfers. All investigators who have studied this phenomenon were able to confirm d'Herelle's basic observations, and it was soon found that lytic bodies, corresponding to other bacteria also, existed in nature. To the entire group d'Herelle applied the generic term *Bacteriophagum*, and he looked upon its representatives as belonging to the class of the filterable viruses and as living organisms. To this opinion he has adhered ever since. While some investigators share his view, others are inclined to look upon the lytic principle as an inanimate agent, and as a product of the bacteria themselves. Doerr (13), Twort, Bordet and Ciuca, Gratia, (14) and others have thus drawn attention to the fact that the lytic agent increases in quantity only during bacterial reproduction, and that when this is prevented, the bacteriophageal titre of the emulsion does not rise. They have shown, moreover, that by starting with a culture of bacteria, devoid of any bacteriophageal properties, these can be made to appear by various artificial means, such as an unfavorable temperature, treatments with immune sera, the mere process of filtration through porcelain filters, etc. These observers have accordingly come to the conclusion that the d'Herelle phenomenon represents an acute infectious disease of the corresponding bacteria, which is due not to a living organism, but probably to an enzyme, furnished by the bacteria themselves, which stimulates the bacteria to renewed formation of this very enzyme, which in turn leads to the destruction of the microorganism.

Observations such as these, if confirmed by further investigations, would be of fundamental importance inasmuch as they suggest new lines of investigation of various diseases, the etiology of which is as yet unknown. We would certainly have to revise our definition of the term infectious disease, which at present implies the activity of an animate agent and the possibility of its transmission from animal to animal, either directly or indirectly. If similar conditions were operative in animals, as apparently are at work in the infectious chlorosis

of plants, various maladies, which now are vaguely classed as metabolic diseases, could be viewed with better reason as infectious processes, and the same would be true for malignant growths. The urgent need of some intensive work along these lines is thus manifest.

3. *The taxonomic position of the animate filterable viruses.* While the possibility cannot be ignored that some of the filterable viruses may be of inanimate nature, there is evidence to suggest that the majority of those, with which we are already more or less familiar, are living organisms. Several of these have been actually cultivated, even though with considerable difficulty, and not without serial breaks, such as the virus of the pleuropneumonia of cattle (Nocard and Roux), that of poultry diphtheria, which is now generally recognized as identical with epithelioma contagiosum (Bordet), the chicken plague virus (Marchoux, Landsteiner and Berliner), the virus of poliomyelitis (Flexner and Noguchi), Olitsky and Gates' supposed causative organism of influenza, and if recent reports are correct, the vaccine virus (Plotz). In the case of the other representatives of the filterable virus group, cultivation has not yet been achieved, but evidences of division have been obtained in corresponding granule formations, which are viewed as the virus proper. Lipschuetz, it will be recalled, suggested the term strongyloplasms for these visible viruses, in contradistinction to those which have not yet been seen with the microscope.

A question that has been asked again and again, is whether the filterable viruses are bacteria or protozoa. To this a definite answer cannot be given. From the fact that an organism can be cultivated in the test tube it does not follow that it must be a bacterium, for many protozoa can be cultivated with equal readiness. To argue for the bacterial nature of an organism on the basis of its form is likewise scarcely warrantable. The fact, therefore, that the virus of small-pox or of poultry pox appears under the microscope as a round granule, is no more evidence that the organism is a bacterium than the capability of locomotion would stamp the typhoid bacillus as a flagellate protozoon. In many viruses belonging to the group of strongyloplasms division has been noted, but a type of division which differs from that of the bacterial cocci. For whereas bacterial division proceeds by direct cleavage, strongyloplasmatic division seems always to be preceded by a gradual process of median constriction, with the consequent formation of dumbbell forms and their gradual separation.

That some of the filterable viruses are more closely related to the protozoa than to the bacteria, is suggested by their peculiar cell tropism and their evident reproduction within the cells and there only. As examples I would recall the remarkable affinity of the variola-vaccinia virus, of the virus of sheep pox, of epithelioma contagiosum, and of hoof and mouth disease for the skin; that of rabies, of poliomyelitis and of chicken plague for the central nervous system; that of pleuropneumonia, of agalaktia contagiosa, of parotitis epidemica for special organs, viz., the lung, the udder and the parotid gland. It is well known that following the intravenous injection of vaccine virus, this rapidly disappears from the circulation and becomes deposited in the skin and there only; the injection of chicken plague virus is similarly followed by its disappearance from the circulation and its deposition in the brain.

Recent investigations have confirmed earlier observations by Novy and MacNeal that certain protozoan parasites (*Trypanosoma lewisi*) may assume a state in which they can pass the pores of antibacterial filters. Reich and Beckwith (15) thus report that they succeeded in infecting guinea pigs with filtrates from the organs and blood of animals infected with *Trypanosoma brucei*.

I would further recall that yellow fever has been produced repeatedly by injecting filtrates of the diluted serum of patients. Reed, Carroll, Agramonte and Lazear (16) first demonstrated this in 1900, and on the basis of these observations came to the conclusion that the causative organism must be of such minute size as to be classed among the "ultra-microscopic" organisms. When Noguchi then suggested that his *Leptospira icteroides* might be the cause of yellow fever, the question arose how this could be reconciled with the demonstrated filterable nature of the virus. It was then suggested that even though the leptospira measured 4 to 9 $\mu$  in length, that its breadth only amounted to 0.2 $\mu$ , and that it is the breadth of an organism which determines its filterability. It was further argued that the organism could pass the filter not only because of its small, transverse diameter, but also owing to its plastic (non-rigid) structure. This may or may not be so, and until leptospiras presenting their usual size and form will have been demonstrated microscopically in the filtrates, the possibility cannot be denied that the organism may be able to assume yet another form and pass in that state.

However this may be, the writer would not suggest for a moment that because an organism, be it a protozoon or a bacterium of the

usually recognized types, can assume a filter passing form, differing from the usual, it should therefore be removed from its proper group and placed in that of the filter passers. More work, however, will have to be done, before it can be definitely accepted that organisms whose normal morphology would warrant their classification either amongst the bacteria or protozoa may under special conditions assume a form which in the past was unknown in connection with the life history of bacteria and protozoa. If further investigations should actually prove the possibility of such an occurrence, a biological explanation would have to be sought. In that event the hypothesis might be advanced that various low forms of animal and vegetable life, while becoming differentiated to higher forms, may yet retain the power to return, temporarily at least, to a more primitive state, with a reduction in size to a point where they would be able to pass the pores of the average antibacterial filter. While such a possibility has already been demonstrated for certain protozoa as shown above, there is evidence to suggest that bacteria endowed with this property also exist. Olitsky and Gates (17) have thus shown that the organism which they have described under the name *Bacterium pneumosintes* may assume a filter passing size, while at other times it may attain a length of one micron. The same writers have also discovered the occurrence in the nasopharynx of four other organisms of the same order which, in contradistinction to *Bacterium pneumosintes*, are non-pathogenic.

While Olitsky and Gates have termed the organism which they regard as the probable cause of influenza, *Bacterium pneumosintes*, they have done so on purely morphological grounds, and have left the question whether or not it really is a bacterium an open one.

To assume, on the basis of our present knowledge, that the filter passers are either bacteria or protozoa, is unwarrantable. We may say that certain observations suggest that a few protozoa, and possibly bacteria may assume a filter passing size, but so far as the great majority of the filterable viruses goes, there is no evidence whatsoever to warrant the claim that their taxonomic position has been established. It might possibly be best to classify them as protista, were it not for the fact that there is evidence to suggest that some of them may be inanimate, even though corpuscular. Theoretically there can certainly be no objection to the formulation of the hypothesis that disease producing agents of corpuscular nature may exist, which in part belong to the lowest forms of life, which are neither bacteria nor protozoa,

and in part to the higher forms of ferments, and that the main group of filterable viruses may properly find its position here rather than either amongst the bacteria or protozoa.

4. *The relation between filterable viruses and specific cell inclusions.* The occurrence of certain cell inclusions which had been noted in several infectious diseases, such as molluscum contagiosum, epithelioma contagiosum and variola, long before the existence of filterable viruses was even suspected, assumed a new significance when it was shown that their appearance was intimately connected with the activity of the infective agent, or what has come to be regarded as the infective agent. Borrell (18) in 1903 was the first to draw attention to the apparent connection between the presence of the infective agent of sheep pox and the presence in the lesions of cell inclusions, resembling those seen in variola, on the one hand, and exceedingly minute granules, which could be stained by the aid of Loeffler's flagellar stain, on the other. In 1907 appeared v. Prowazek's classical study (19) of the various cell inclusions which had been noted up to that time, viz., the three mentioned above, Borrell's sheep pox bodies, the Negri bodies of rabies and the trachoma bodies which v. Prowazek had discovered himself in association with Halberstaedter. On the basis of his studies of these various structures, and especially of the trachoma bodies, which at a certain stage of their development could be shown to consist of innumerable tiny little granules, v. Prowazek suggested that all these inclusions are composed of virus granules proper, surrounded by a mantle of reaction products furnished by the cell which has been invaded by the virus. To express this concept of their structure, he suggested the term *chlamydozoa* for this group of the filterable viruses. The suffix, *zoa*, was chosen to express his belief that the viruses in question belonged to the group of protozoa or were more closely related to these than to the bacteria. Lipschuetz (20), it will be recalled, proposed the term *strongyloplasms* for all viruses of the filterable type, which can be seen through the microscope, irrespective of their association with reaction products. That v. Prowazek's interpretation of the meaning of these cell inclusions has in the main been correct, has now been abundantly demonstrated. This discovery of course, has shown a new direction in which research might profitably be carried on in the search for as yet unknown causative agents of diseases of apparently infectious nature, for the presence of cell inclusions now suggests the activity of a filterable virus. It will be well to bear in mind, of course, that the absence of cell inclusions in a given

disease does not imply the absence of a filterable virus as the etiological agent. But it also goes without saying that even though inclusions have in the past not been noted in the specific lesions produced in the course of a disease, it does not follow that they may not be present after all, that they merely have been overlooked in the past. A great deal of reinvestigative work will have to be done in the various infectious diseases in which the cause is as yet unknown, before we can definitely exclude the activity of a filterable virus. That such a reinvestigation may be crowned by success, has already been shown. Lipschuetz (21) thus was able to demonstrate the presence of cell inclusions in the lesions of all three types of herpes, viz., herpes simplex (febrilis), herpes genitalis and herpes zoster, which prominent investigators in the past had evidently either overlooked or failed to recognize. That these inclusions are in reality specific, and that Lipschuetz's interpretation of their nature is correct, is shown by the fact that the identical structures appear in the corneal epithelial cells of the rabbit, following inoculation with the contents of the herpetic vesicles. Their presence, moreover, is confined to the actual seat of the lesions which develop as a consequence of the inoculation; in herpes simplex and herpes pro-genitalis they have been shown to appear in serial inoculations, and in the case of herpes simplex the virus has been shown to be filterable. In this connection it may be interesting to note that whereas the majority of cell inclusions appear in the cytoplasm of the affected cells, the herpetic inclusions occur practically exclusively within the nucleus. Similar inclusions, occurring both in the cytoplasm and nucleus have been found by the same observer in paravaccinia (22). The latter term has been applied to an anomalous response to vaccination with calf lymph, which so far as known has only been observed in Austria, or more specifically in Vienna. The opinion has been advanced that in some manner some of the calf lymph produced at one of the vaccine institutes of that country has become contaminated by an aberrant form of vaccine virus, and that this has been propagated together with the latter. If this be so, it is remarkable that the resultant cell inclusions should be so different from the Guarnieri bodies of vaccinia. It is true that the latter may also appear within the nucleus, but that is after all unusual.

In yet another disease of doubtful etiology cell inclusions have been described within recent years which, if confirmed, would suggest the possibility that this disease also may be due to a filterable virus. The malady in question is known as *Verruga peruviana* or Peruvian warts,

and apparently represents a later phase of so-called Oroya fever. The inclusions in question were discovered by da Rocha Lima (23) in endothelial cells of the corresponding lesions and are composed of minute granules, which like the majority of similar structures, can only be satisfactorily demonstrated with Giemsa's or Levaditi's method of staining.

Still more recently Gins (24) has drawn attention to the occurrence of intranuclear inclusions in the epithelial cells and cells of the underlying corium in the specific lesions which develop in guinea pigs after inoculation with the virus of hoof and mouth disease. It will be recalled that the disease in question was the first infectious disease affecting animals in which a filterable virus could be shown to be the causative agent (Loeffler and Frosch (3) 1898). Cell inclusions have in the past been reported in the skin and the corresponding lesions affecting the mucous membranes by several observers, but none of these have proven to be specific. Whether the inclusions described by Gins are specific will have to be determined by future investigations. Inasmuch as the recognition of the disease must at present be based upon the progress of the malady or on the result of animal inoculations, which means a considerable loss of time, the possibility of reaching a prompt diagnosis by microscopic examination would prove of great advantage.

RECENT INVESTIGATIONS ON THE RÔLE OF FILTERABLE VIRUSES AS DISEASE-PRODUCING AGENTS. *a. The supposed relationship of the herpetic virus to the causative agent of epidemic encephalitis.* In the foregoing section we have already pointed out that specific cell inclusions are demonstrable in herpetic lesions (21) and that this fact in itself may be regarded as implying the activity of a filterable virus as the causative agent. The proof of the correctness of this conclusion is complete so far as herpes simplex goes, while in the case of herpes zoster and herpes genitalis the evidence, though strong, is as yet circumstantial.

Renewed interest in the etiology of this group of dermatoses was aroused by Grueter's discovery (25) that the inoculation of the rabbit cornea with scrapings from lesions of dendritic keratitis produces a violent inflammation which can be transmitted from animal to animal. Loewenstein (26) and later Luger and Lauda (27), confirmed these observations and further showed that the inoculation of the rabbit cornea with the contents of herpes febrilis vesicles, from whatever source, led to the same result. While Loewenstein did not succeed in infecting animals with material that had been passed through a

Berkefeld filter, he concluded nevertheless from certain microscopic findings that the pathogenic agent probably belonged to the chlamydozoa. Subsequent positive filtration experiments, on the part of Luger and Lauda (27), Blanc and Caminopetros (28), Levaditi, Harvier and Nicolau (29) have confirmed the correctness of this conclusion.

Aside from the occurrence of the virus in the herpetic vesicles it would seem that the same or a closely related virus may occasionally also be encountered in the saliva of normal individuals.

The discovery of the virus of herpes febrilis or, as it would no doubt be better to say, of herpes simplex, while of great interest in itself, has attracted special attention for the reason that several investigators have reported the finding of a virus in material obtained from cases of epidemic encephalitis which seems to be identical with it, or at least very closely related to it. Doerr and Schnabel (30) thus report that rabbits which had recovered from keratitis produced by the common herpes simplex virus and which had developed an immunity to reinoculation with it, were also resistant to their encephalitic strain, even when introduced subdurally. Levaditi, Harvier and Nicolau (29), as well as Blanc and Caminopetros (28) did not obtain the same, but very similar results. They found that their supposed encephalitic virus was more virulent than their herpetic virus, and that while animals which had become immune to the former were also immune to the latter, the converse did not hold good. As a matter of fact a certain proportion of rabbits which have been inoculated with the common herpes virus, after a variable length of time develop symptoms of encephalitis and usually succumb, and post-mortem lesions are demonstrable in the brains of such animals which are very similar to those observed in the human being. The idea that a filterable virus may be the cause of epidemic encephalitis was originated by Strauss, Hirschfeld and Loewe (31) in 1919. These observers reported that the virus of epidemic encephalitis will pass Berkefeld N filters, that it may be preserved in glycerin, that it can be cultivated in Noguchi's spirochetel medium; that it can be stained with Loeffler's flagellar stain and Giemsa's stain; that rabbits and monkeys can be successfully infected with cultures by the subdural route, and that the same symptoms and pathological changes can be produced with these cultures, as by direct inoculation of brain substance, naso-pharyngeal mucous membrane, spinal fluid and naso-pharyngeal secretion from human cases of the disease. They further reported that animal passage could be effected without much difficulty. Their results with naso-pharyngeal

washings were indeed so uniform that they suggested the animal experiment as a means of diagnosis.

It is noteworthy that other investigators have been far less successful than Strauss, Hirschfeld and Loewe in transmitting the supposed virus of epidemic encephalitis to experimental animals and, as matters now stand, it seems rather doubtful whether the virus of any one of the experimenters referred to above actually represents the causative agent of epidemic encephalitis.

Kling (32) in a series of recent communications, has reported that on inoculating rabbits by the subdural route with brain material from cases of epidemic encephalitis, an infection does develop which pursues a latent course, however. A few animals died after from one to three months. The remainder were killed after four to six months. In contradistinction to the findings of Doerr, Levaditi and others, Kling found changes of a more chronic character, in as much as in the meninges and in the perivascular and focal infiltrations of the brain itself, mononuclear elements predominated. The lesions, moreover, were found to affect the midbrain rather than the cortex. Kling emphasizes the identical character of the lesions in his animals with those occurring in the human being. He further points out that his virus produces no lesions in the rabbit cornea that are at all comparable with those which follow inoculation with the herpetic virus, and that no crossed immunity was demonstrable between the two types. He concludes that his virus is the true cause of epidemic encephalitis, while that of Levaditi, Doerr and their collaborators is merely the herpetic virus which has accidentally found its way to those regions where it has been found.

*b. Bacterium pneumosintes and its relation to epidemic influenza.* Up to the beginning of the great pandemic of influenza of 1918-1919, Pfeiffer's bacillus had been fairly generally regarded as the probable causative agent of that disease. The inconstancy of its occurrence during this epidemic, however, made the correctness of this view appear rather doubtful. The inconstant presence of various other bacteria also, which were encountered both *intra vitam* and *post mortem*, eliminated these as specific factors. The thought hence suggested itself to many observers that a hitherto undiscovered organism might be the cause of the epidemic disease, and that Pfeiffer's bacillus, like the various streptococci and staphylococci that are so frequently present, merely represent secondary invaders or symbionts. With this idea in mind Olitsky and Gates (33) undertook the study of the

naso-pharyngeal washings of cases of uncomplicated influenza with reference to the presence of such as yet unknown factors. The initial studies were begun while the epidemic wave of 1918-1919 was in progress, and have been continued up to the present time. By inoculating rabbits through the trachea with the washings from the nasopharynx of uncomplicated cases of influenza, during the first thirty-six hours of the disease, Olitsky and Gates obtained clinical reactions (fever and leucopenia, with mononuclear depression) and pathological changes in the lungs of their animals, which resembled the findings in human influenza. By injecting the lung juice from such animals into others the same results were obtained. Further investigations then revealed that the virus in question readily passed through Berkefeld V and N candles, and that when contained in bits of lung tissue, it withstood the action of a 50 per cent solution of glycerin for a period of nine months. Especially significant also was the observation that it produced an effect upon the lung tissue of rabbits which favored the invasion and infection of the lung with various bacteria, such as pneumococcus, type IV, and atypical type II, streptococci and Pfeiffer's bacillus. Attempts at cultivation showed that by starting with filtered naso-pharyngeal secretions of patients, or unfiltered or filtered lung tissue suspensions from infected rabbits growth could be obtained under strictly anaerobic conditions in a medium composed of sterile human ascitic fluid, and a fragment of fresh rabbit kidney. The organism in question, as obtained in primary culture, is a minute bacilloid body, measuring only 0.1 to 0.3 $\mu$  in length, the breadth being approximately one-third of the length. In subcultures on media consisting of meat infusion—peptone broth or agar as a base, and enriched with fresh animal or vegetable tissue and especially in the presence of dextrose, larger forms up to 1 $\mu$  in length may be seen.

Identical findings were obtained during the outbreak of 1920 and again during that of 1922. The clinical and pathological effect in the inoculated animals was the same whether filtered or unfiltered nasopharyngeal washings, or filtered or unfiltered lung juice, or filtered or unfiltered cultures were used. Negative results only were gotten in the case of healthy individuals and persons not suffering with influenza, as well as with animal control material. The conclusion thus seems justifiable that the organism described by Olitsky and Gates probably represents the actual cause of epidemic influenza.

Regarding the nature of the virus in question, the writers are somewhat noncommittal. They recognize that their organism is "not of

the nature of ordinary bacteria" (IV, 728), and express the belief that it is a representative of a "group or class of minute microorganisms, which the anaerobic Smith-Noguchi technique has thrown open to exploitation." If nevertheless they termed the organism *Bacterium pneumosintes* they did so merely on the basis of its bacilloid form, the species name having reference to its injurious action upon the lung tissue.

In this connection it may be of interest to note that in the course of their studies of naso-pharyngeal washings, and using the same anaerobic technique, Olitsky and Gates met with a number of other filter passers which in contradistinction to *Bacterium pneumosintes* were non-pathogenic for rabbits.

It is clear that a very interesting field has here been opened, irrespective of the question what the taxonomic position of the organism in question may be. From the bacteria that we have known in the past, the organisms differ in at least two important particulars, viz., their minute size and the remarkable resistance which they offer to the action of glycerin. In both respects, as well as in its case of dissemination, *Bacterium pneumosintes* strongly resembles the causative agent of smallpox.

*c. Recent investigations into the etiology of measles.* Although many attempts have been made in the past to isolate the causative agent of measles by the usual bacteriological methods, it cannot be said that any one of the various organisms which have been found, has yet been proven to be the specific cause of the disease in question. Elevation of temperature, various exanthems and enanthems and leucocytic drops have been noted following the injection of various organisms obtained from the blood and the naso-pharyngeal secretion of measles cases, but the very fact that such reactions occur inconstantly and have been obtained with different organisms, suggests that none of them can be regarded as specific. If it be remembered how readily the measles patient falls a prey to secondary invaders, notably representatives of the streptococcus family, it cannot be surprising that organisms of this order may be found, nor that corresponding antibodies should appear in the blood.

One great difficulty in the study of the etiology of measles has been that whereas blood, taken during the immediate pre-eruptive and early eruptive stage, when injected into children even in so minute an amount as 0.002 cc., produces the typical disease (34), we have not yet found an animal in which the identical clinical picture can be produced.

Significant, however, is the fact that, as Goldberger and Anderson (35) first showed in 1911, the blood and naso-pharyngeal secretion of measles patients, even after filtration through Berkefeld candles, will produce symptoms in monkeys, which at least are suggestive of the human disease. Goldberger and Anderson found that after a period of incubation, varying from three to twenty-one days, there followed a febrile period of from four to five days, and frequently an exanthem appeared three days after the onset of the fever. This varied in color from a rose red to a coppery hue and was maculo-papular in character. Scaly desquamation then followed, and after recovery the animals were immune to reinoculations. This clinical picture was transmitted through the blood through six successive groups of animals, and healthy animals contracted the disease by living in contact with the sick ones.

These results were confirmed by Nicolle and Conseil (36), Hektoen and Eggers (37) and still other investigators.

Sellard and Wentworth (38) objected to the interpretation of these findings, however, claiming, that the symptoms produced were merely symptoms of serum sickness, and that they obtained similar results by injecting normal human blood serum.

The validity of this objection falls to the ground, however, if it be remembered that Goldberger and Anderson obtained the same results in serial inoculations. Nicolle and Conseil, moreover, furnished unquestionable evidence that the clinical picture in injected monkeys is directly connected with the transfer of the virus. They injected a monkey with measles blood. After nine days the animal developed fever; two other monkeys were then inoculated with the blood of the first animal, and reacted in the same manner. At the same time a child was inoculated with the blood from the first monkey and developed typical measles. The blood of this child was injected into three monkeys, and of these two reacted with fever, as did the first.<sup>1</sup>

Blake and Trask (39) disposed of the validity of Sellard and Wentworth's objections in another manner. They used no blood at all, but injected naso-pharyngeal washings intratracheally. Of ten monkeys which were tested in this manner, eight developed symptoms suggestive of measles, one gave a doubtful result, and one died before the expiration of the period of incubation. The same results were

<sup>1</sup> It should be stated that Nicolle and Conseil's animals developed no eruption, which is contrary to the findings of Goldberger and Anderson, who noted this often, though not in all cases. Possibly this may be due to the fact that different species of monkeys were used.

obtained with the naso-pharyngeal washings, after filtration through Berkefeld N candles, and followed spraying of the nose and throat, as well as after intratracheal inoculation. The animals which recovered had become immune to a repetition of the treatment. Animal passage was also secured, so that the argument that a soluble toxin may have been responsible for the clinical picture can be ruled out.

It is to be noted that in Blake and Trask's series the clinical picture seemed to be quite constant, and it is noteworthy that both exanthems and enanthems developed, and that the histological examination of both showed the same lesions as are found in the human disease.

Inasmuch as monkey material is expensive, and the possibility of investigating problems connected with the subject of measles would thus be limited, renewed attempts have been made to infect the ordinary laboratory animals, notably guinea pigs and rabbits, with the measles virus. It was to be expected that any clinical symptoms that would be produced, would probably or at least possibly be even less characteristic and less constant in these animals than in monkeys, and for this reason some of the investigators sought to ascertain whether other effects might not be noted, which could serve as criterion of successful infection. Results of definite value have, as a matter of fact, been achieved along these lines. It has thus been shown that rabbits are quite susceptible to the action of the measles virus, both when introduced in the form of blood or of filtered naso-pharyngeal secretion, if obtained early in the course of the eruptive period.

Nevin and Bittman (40) found that in nearly all of their animals, which had been inoculated with blood, definite clinical symptoms developed between the thirteenth and seventeenth day following the inoculation, such as conjunctivitis with moderate edema of the lids, a little diarrhea, a slight exanthem, followed by desquamation in the shaved axillary and thoracic regions, and sometimes hyperemic spots on the labial mucosa. Nevin and Bittman report that in their series fever was inconstant. Especially interesting was the fact that the same results were obtained in a second series of animals, which had been inoculated with blood from the first series.

Duval and d'Aunoy (41) obtained similar results. They report, however, that the period of incubation was only of two to five days' duration; pyrexial and cutaneous as well as leucocytic reactions appeared. The rise in temperature took place on the fourth day and concomitantly with it there occurred a diminution of the total number of the leucocytes. While 90 per cent of their animals gave a pyrexial

and leucocytic response to the inoculation, only about 40 per cent developed a rash. Coryza, conjunctival injection and enanthems on the buccal mucosa of the oral cavity were common symptoms. Repeated passage through rabbits seemed to increase the virulence of the virus. A number of animals thus succumbed in the fourth and subsequent generations, and in these grave nephritic changes could be demonstrated at autopsy. A similar pyrexial and leucocytic response was obtained in guinea pigs, but in these animals the incubation period was longer, viz., nine to twelve days. Nephritis was a constant symptom. Cross passages from rabbits to guinea pigs and vice versa, were successful, and the guinea pigs which had reacted and recovered were not susceptible to reinoculation with measles blood when tested over periods of two weeks to three months after recovery.

Corresponding results were obtained by Grund (42) and Duval and d'Aunoy (41) with the filtered naso-pharyngeal secretion of measles patients. Curiously Grund, in rabbits, was unable to effect animal passage by starting with material taken from the living animals, but did succeed if the inoculations were made with the blood and lung juice of animals that had succumbed to experimental measles.

On the basis of the experimental work that has thus far been done the conclusion would seem to be justifiable that the measles virus is a filter passer, that it is present in the blood and naso-pharyngeal secretion of patients during the early eruptive stage of the disease, and that rabbits and guinea pigs may properly be used for the propagation of the virus in the laboratory and the study of its properties. Such investigations are now urgently needed, and should determine the rôle which Tunnicliff's green producing diplococcus (43) plays in the etiology of the disease.

*d. Trachoma and inclusion blenorrhea.* The search for the etiological agent of trachoma has been attended by great difficulties, owing in large part to the remarkable tendency of the disease to become complicated by various bacterial infections. These infections have misled various investigators into the belief that the particular organisms which they happened to encounter with some degree of regularity represented the cause of the malady. In every instance further investigations have demonstrated that the suspected bacillus or coccus was merely a concomitant organism or a secondary invader.

In 1907 v. Prowazek and Halberstaedter (44) made the discovery that peculiar inclusions may be found in the epithelial cells of the diseased conjunctiva of both human patients and orang-utangs that

had been inoculated with trachomatous material. The study of the structure of these inclusions led v. Prowazek to the development of his chlamydozoal doctrine and the conclusion that in this particular instance the constituent granules of the inclusions represented the etiological agent. Cell inclusions, in the sense of v. Prowazek, it will be recalled, signify the presence of a filterable virus which, after invading a cell, calls forth the deposition of a reaction product about the individual granules.

That the virus of trachoma is in reality filterable has been shown by Bertarelli and Cechetto (45) as well as by Nicolle, Cuénod and Blaizot (46).

Noguchi and Cohen (47) claimed to have cultivated the virus in question, but it is to be noted that they were unable to produce typical lesions with the cultures. In the same year (1913) Halberstaedter and v. Prowazek announced that they had observed an increase in the number of inclusions in the epithelial cells, which they had attempted to cultivate.

A fair degree of headway seemed thus to have been made in the solution of the problem of trachoma, when Heymann (48) announced that he had met with similar inclusions in several cases of gonorrheal blenorrhea neonatorum, and expressed the opinion that they represented a specific reaction to the gonococcal virus. A thorough study of this question then led to the interesting discovery of the existence of an inclusion blenorrhea as a malady *sui generis*, which primarily affects the genitalia of both male and female and secondarily the eyes of the new-born. This type of blenorrhea it is now known may be associated with a gonococcal infection, as well as with other bacterial infections (pneumococci, diphtheria bacilli), but when this occurs the processes are independent of each other.

The discovery of the occurrence of inclusions in connection with blenorrhea of this type naturally threw doubt upon the correctness of v. Prowazek's view, that the constituent granules making up the inclusions found in trachoma actually represented the trachomatous virus. Various suggestions have accordingly been made to account for their appearance in trachoma, on the one hand, and in inclusion blenorrhea, on the other. Lindner (49) did not hesitate to declare the two processes as identical, for in two baboons which he inoculated with pure inclusion blenorrheal material he claims to have obtained a clinical and pathological picture which could not be distinguished from trachoma. Wolfrum (50) even went so far as to inoculate the secretion

from a pure case of inclusion blenorrhea neonatorum into a normal eye of a human being, with the consequent development of the typical picture of trachoma. These two experiments would seem to settle the question of the identity of the virus of trachoma and inclusion blenorrhea were it not for the fact that ophthalmologists do not seem to agree in their opinion whether the diagnosis of trachoma can be made with certainty during the early stages of the malady, i.e., at a time when pannus and scar tissue formation have not yet developed, and when granules represent the sole lesion.

One point which has struck the writer as rather peculiar is that inclusions in trachoma supposedly can only be demonstrated during the very earliest stages of the disease, and that later on they are absent, the inference being that when they cannot be found the condition must be viewed as having passed beyond the earliest stage. This, however, is not always true, and one asks oneself whether Heymann may not be correct in assuming that trachoma is after all a disease *sui generis*, the cause of which is still unknown, and that those cases, where inclusions are demonstrable, represent a double infection, viz., of trachoma with inclusion blenorrhea superadded, and that the inclusions really do not belong to the picture of trachoma. A great deal of work is evidently yet to be done before this problem is solved, and it is rather surprising that during the past ten years so few investigations in this direction have been undertaken. If the enormous importance of trachoma as a public health problem and a source of human misery in so many parts of the world be borne in mind, this should constitute a sufficient impetus for prompt and active investigation, aside from its interesting scientific aspects.

*e. Filterable viruses and plant and insect diseases.* In conclusion we would briefly refer to the growing interest which the filterable viruses have elicited amongst plant pathologists and entomologists.

It will be recalled that the existence of the filterable viruses was first discovered in a plant disease, viz., the so-called mosaic disease of tobacco, the economic importance of which is sufficiently attested by the fact that the United States Department of Agriculture in its Bureau of Plant Industry maintains a special division in which the etiology and measures of control of this disease are continually being studied. Doctor Allard's name deserves special attention in this connection, for he has unquestionably done more than any other investigator in elucidating the nature and properties of the virus and its mode of dissemination (51).

The demonstration of the connection of a filterable virus with the mosaic disease of tobacco has naturally led to a study of other diseases which affect plants, that are of interest to the agriculturalist, such as the mosaic disease of cabbage, of corn, of Irish potatoes, of cucumbers, of beans, of sugar corn and other grasses from a like angle. The initial studies which have thus far been made clearly show the enormity of the problem.

It has been shown already that in the dissemination of these diseases in the plant world insects probably play a very important rôle, and an investigation of the viruses within the insects has thus suggested itself as yet another field for the study of these interesting disease-producing agencies.

That insects in turn may not merely act as transmitters of filterable virus diseases to plants and animals (e.g., Pappataci fever, dengue, catarrhal fever of sheep, etc.), but may themselves fall a prey to their activity, has been established in several instances, viz., in connection with the so-called polyhedral disease (52) (jaundice) of silk worms (v. Prowazek), the wilt disease of the European nun moth (53) (Wahl), and the American gypsy moth caterpillars (R. Glaser) (54) and the sacbrood of bees (White) (55). So far as we are acquainted with the filterable viruses, they are destructive or at least harmful to their hosts, but that some of them may also prove of benefit, to men at least, is indicated by the fact that the appearance of wilt disease amongst gypsy moths has probably done more to bring about the eradication of this pest in this country than all attempts at control by man.

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## MINERAL METABOLISM IN RELATION TO ACID-BASE EQUILIBRIUM

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Liebig's researches in 1840 (125), (126) on the minerals in plants and in animal tissues were the first scientific approach to the problem of mineral metabolism. Then in rapid succession came the analyses of bone by Bibra, in 1844 (9); the discovery of the alkaline tide of the urine by Bence Jones in 1850 (105); studies on the effects of acids and alkalis on mineral excretion by Buchheim and his students at Dorpat, (29), (41), (150), (236) in 1850-1865; and the researches of Bunge (25), (26) who worked more than twenty years on the composition of blood and milk and the action of salts. Soon the problems of mineral metabolism spread to many fields. Reviews of the early literature have been made by Voit in 1882 (227), and Loewi in 1907 (129), (130). In 1906 Albu and Neuberg (3) published the first monograph on the subject. A monograph by Forbes and Keith (49) contains references to all the aspects of metabolism studies in which phosphorus plays a part. Matill and Matill (140) have made the latest review of mineral metabolism.

In recent years investigations emphasize the physiological importance not only of minerals but also of the resultant hydrogen ion concentration to botany and soil chemistry, to muscle contraction, nerve irritability, cell permeability and bacterial growth. In metabolism the new type of biological experiments—feeding animals with purified substances—necessitated the careful consideration of salts in food. New studies have also clarified problems in acidosis and alkalosis. Data obtained from these various sources have renewed interest in mineral metabolism of human beings.

Most studies have been fragmentary—each investigation has dealt with one or several mineral constituents. A few complete studies were made, but even from these no conclusions have been drawn as to the significance of mineral metabolism in acid-base economy. In order to recognize this relationship, intake and output of salts must

be calculated in common terms; namely, as normal solutions. The analogy to energy metabolism is obvious. Salts can be evaluated in cubic centimeters of normal solution just as grams of protein, carbohydrate and fat can be reckoned in calories. The calcium requirement can be expressed in cubic centimeters of normal solution or per cent of the total cubic centimeters of salt, just as the protein requirement can be stated in calories or in per cent of the total calories. Given the total requirement, the extent to which salts are interchangeable must be determined for the various radicals, just as isodynamic quantities of fat and carbohydrate can be calculated. Amounts of minerals can be compared directly as chemical equivalents. It can thus be determined whether food and excreta are acid or basic and whether the metabolism has resulted in a gain or loss of acids or base.

Mineral metabolism studies must be recalculated in terms of acid or base. A measurement of intake and output of a substance shows the "balance." When acid or base is retained, the balance is positive; when excreted in excess of intake, the balance is negative; and when the body neither gains nor loses, it is said to be in acid-base equilibrium. The value of acid or basic solutions can be stated in cubic centimeters of normal solutions—plus sign for acid or minus sign for base. For purposes of the computation of analyses in terms of acid or base, sodium, potassium, calcium and magnesium form the "base" value; sulphur, chlorine and phosphorus, the "acid" value. Sodium, potassium and chlorine are monovalent; calcium, magnesium and sulphate are divalent. Phosphorus is calculated by Sherman as divalent, but in the body it is 1.8. The algebraic sum gives the excess of acid or base. Such problems, as the fractions of the phosphorus and sulphur which are burned to form acid radicals, make accurate computation difficult. In stating the value of acid-base metabolism, it is convenient to speak not of acid balance, but always of "base balance." The acid or base which is lost or retained gives rise to four possibilities.

1. Base retention = positive base balance
2. Acid excretion in excess of intake = positive base balance
3. Base excretion in excess of intake = negative base balance
4. Acid retention = negative base balance

From the many excellent metabolism studies, those have been selected in which all the acid and basic elements have been determined in food, urine and feces and which, therefore, permit critical analysis of acid-base economy. Elements which have not an important acid or base value—as iron, iodine, fluorine, manganese, arsenic, silicon, etc.—will

not be covered in this paper. Though they may be essential for life processes, they occur in such minute amounts that for metabolic experiments their acid or base value can be neglected. We shall discuss mineral metabolism from five main viewpoints: 1, acid-base requirements; 2, acid-base metabolism; 3, acid-base equilibrium in the body; 4, the effect of acid on mineral metabolism; 5, the effect of alkali on mineral metabolism.

**ACID-BASE REQUIREMENTS.** That the body requires minerals for carrying on its functions was clearly stated by Bence Jones in 1850, (105, p. 3):

Some idea of the comparative value of different classes of substances is obtained by noting the rapidity with which animals die when any one of them is absent from their food. Thus, the following order might be formed, 1st, air; 2nd, water; 3rd, organic substances containing nitrogen; 4th, organic substances free from nitrogen; 5th, inorganic substances, the ashes, salts.

Further, (p. 7):

With white of egg . . . the animal fed on it alone died of starvation. The same results follow from a diet of sugar or fat alone; and, if nitrogenous and non-nitrogenous food was given perfectly free from all saline matter, perfect nourishment of the body could not take place. Still more than this, if one single salt which exists in the body was left entirely out of the food, disease, if not death, results.

*Salt starvation.* The above statements are clear but lack convincing experimental proof; they were given weight by the classic experiments of Forster (52). He showed that diets which were salt free but otherwise adequate caused death.

Pigeons, which were fed with salt poor diets, died in thirteen, fifteen, and twenty-nine days; two dogs likewise fed, after twenty-six and thirty-six days, were near death. Because the animals were in nitrogen equilibrium, death can only be attributed to the lack of the ash materials in the diet. The intake of these should not fall below definite limits. With the most complete removal of the minerals in the diet of grown animals, the processes of metabolism, catabolism, and destruction go on in the same way as in the usual diet, adequate in all respects. Still profound disturbances in the function of the organs come about, which hinder changes of the food material into absorbable modifications and the substitution of destroyed body materials. In part the processes necessary for life are depressed before the impossibility of a lasting food absorption draws destruction and death after it.

Although the dog remained in approximate nitrogen equilibrium on a diet of meat powder, fat, and starch, the salt content of excretion was small but was always greater than the intake. Stupidity, loss of interest, trembling, muscle weakness, paralysis of the hind legs and later convulsions and raging developed.

Although the body contained probably 1500 gm. of salts at the onset, this condition entailed the loss in feces and urine of only 30 gm.  $P_2O_5$  and 7 gm. NaCl in twenty-six days.

Bunge felt this might be due to the production of sulphuric acid in the metabolism of the protein. At his suggestion, therefore, Lunin (132) repeated these experiments on mice, using a diet containing only 0.06 per cent of ash. To this he added  $NaHCO_3$ . The animals receiving the alkali lived longer than the controls, but died showing similar symptoms.

Taylor (220) repeated these experiments on himself. He took a diet consisting of 70 grams of washed coagulated egg white, 120 grams of washed fat and 200 grams sugar—comprising 2250 calories—and 1 gram ash. There was nitrogen equilibrium; the sulphur in the urine was fairly constant and of such magnitude as to indicate that it came from the food. Phosphate excretion reached a constant level of 0.8 gram  $PO_4$ , chlorine fell to 0.2 gram per day, and calcium and magnesium disappeared from the urine after the fourth day. The acidity of the urine was measured by the hydrogen electrode but the value is probably incorrect. The urine is too acid for the accompanying ammonia, which stayed constant. On the ninth day acetone was observed on the breath and the experiment was discontinued. Taylor describes his main complaint as loss of appetite; his nervous symptoms were soreness in the muscles, with normal reflexes and circulation. There was marked diaphoresis and diuresis. He lost 1.5 kgm. of weight but this was regained as soon as the experiment was discontinued. Since the feces were not analyzed, it is impossible to compute the balances.

Goodall and Joslin (70), working under Folin's direction, repeated this experiment on two students, giving attention to the fractions of nitrogen and sulphur. They kept one man on the diet for thirteen days and the other for nine days. They confirmed Taylor's experiments in all particulars, except that no acetone was formed. They also failed to analyze the feces, so that no data are available as to the minerals lost in metabolism. They also bring out the fact that there was rapid loss of weight. One man lost 2.5 kgm. in the first three days and 2.5 kgm. in the next ten days. In the seventy-two hours after the experiment was discontinued he gained 4.1 kgm.

V. Wendt (234) has studied his complete mineral metabolism during fourteen days for 1st, a diet low in both ash and protein; and during twelve days for 2nd, a diet low in ash and liberal in protein. These are not reported in detail because he made a series of salt additions

which, therefore, make interpretation difficult. To calculate the base balance is also difficult since he does not give the separate values for sodium and potassium.

This remarkable group of experiments should be repeated and the total mineral metabolism determined. The conditions in salt starvation differ from those in fasting; in salt starvation no body protein is destroyed, with consequent acid production. Hence the acid-base value of the excretion should be different.

*Fasting.* Benedict (8) determined all the acids and bases in the urine of a fasting man over the whole period of thirty-one days and, as there were no stools, the values represent the total excretion. Com-

TABLE 1  
*Benedict's fasting man—Average excretion per day*

	cc. 0.1 N		cc. 0.1 N
Cl.....	111	Ca.....	105
P.....	465	Mg.....	55
S.....	355	K.....	228
		Na.....	114
(1) Acids.....	+931		
(2) Bases.....	-502		
(3) = (1) - (2) Excretion.....	+428		-502

puted on the basis of normal solutions, as shown in table 1, the excretion represents a daily average for thirty-one days of 931 cc. 0.1N of acids radicals, 502 cc. of alkali radicals, or an excretion of acid of 428 cc. 0.1N. For the total period, 13,268 cc. 0.1N acid were excreted from an alkaline blood.

In fasting the great loss in weight is largely due to the loss of fluids. There is also a breakdown of body substance. Mineral excretion represents the sum of these two. Gamble (1921) has taken into consideration this loss of "body water" as a source of mineral output. The data, when published, will permit calculation of the acid base metabolism. The loss of body fluids in salt starvation and fasting indicates the practical importance of acid-base metabolism in anhydremia.

*Normal requirements for adults.* The need of minerals can be ascertained from dietary studies of freely chosen dietaries. The first study of this type was that of Sherman on iron (197). Shortly following appeared that of Sherman, Mettler and Sinclair (198), of

Tiegerstedt (221), and of Horneman (91) on calcium, magnesium and phosphorus.

Sherman has completed his original purpose of analyzing diets for all the elements and has given the average intakes of the mineral constituents of 150 American dietaries (199, p. 271) calculated on per man per day and per 3000 calorie basis. The minimum is probably something below, and the average above, the actual requirement. These can be compared with data he has more recently published, derived from metabolism experiments (204); the calcium requirement, average of 97 studies, is 0.45 gram, or 225 cc. per man per day (201) and the

TABLE 2  
*Sherman's data for minerals in food, calculated as normal solutions*

ACIDS			ALKALI		
	cc. 0.1 N			cc. 0.1 N	
	Mini- mum	Aver- age		Mini- mum	Aver- age
P.....	418	925	Ca.....	175	365
Cl.....	233	810	Mg.....	140	283
S.....	498	813	K.....	418	870
			Na.....	96	850
(1) Acids.....	+949	+2548			
(2) Bases.....	-829	-2368	Bases.....	-829	-2368
(3) = (1) - (2) Food. ....	+120	+180			

phosphorus requirement, average of 95 studies, is 0.88 gram, or 464 cc. 0.1N. Sherman's figures in terms of acid or base value per 3000 calories per day are recalculated in table 2.

Blatherwick (17) has by similar calculations found in thirty-two army dietary studies an average of 22 cc. 0.1N acid per man per day, with variations from 494 cc. of acid to 250 cc. of alkali. Since these diets were adequate in other respects and the men thrived upon them, it is only fair to assume that other freely chosen mixed diets would vary as widely in acid-base values when studied over short periods. Because Sherman's data are so well checked by metabolism studies, they more accurately represent the needs of the body. The normal requirements of an adult are, therefore, approximately 150 cc. 0.1N acid per day.

ACID-BASE METABOLISM. Dietetic studies on large groups to determine the normal consumption of minerals give us no insight as to

their use in the body. The changes which take place can be determined only roughly. Inadequacy of the diet is shown by gross pathological changes or death. The acid-base metabolism can be calculated from suitable data in the literature for 1, fetus; 2, infant; 3, child; 4, adult; 5, women during pregnancy and lactation.

*Fetus.* For the fetus the problem of mineral metabolism is simplified. We cannot estimate the intake and output, but can only take into account the balance. The balance is, of course, positive for all elements and represents the retention of minerals during growth. Extensive work has been done on ash analysis of the fetus and the newborn. There is an excellent review of this material in Czerny and Keller (32), chapter 14. The original object of much of the work was to determine whether Bunge's theory that the ash of the fetus resembled the mother's milk rather than the mother's blood could be substantiated. The facts did not bear out this theory—and Bunge was led to modify the theory to the following—that the time required for the fetus to double its weight was dependent upon the composition of the mother's milk. However, much valuable material has been collected.

The data show the relation of the growth of the fetus to its mineral metabolism. The weight and ash analysis of the fetus are given by Fehling (44) and Camerer (27). The percentage of ash increases from 0.001 per cent at six weeks to 3.3 per cent of total weight at term. As the measurements and weights of the fetus are given, the total ash can be calculated and equals at four months 0.369 gram; at five months, 1.33 gram, at six months, 6.0 grams; at seven months, 26.6 grams; and at term, 100 grams. The percentage of ash and the total value of ash before four months is so small as to be negligible in the maternal metabolism. There is an actual gain in the weight of ash from the fourth to the fifth month of 1.0 gram, from the fifth to the sixth of 5.6 grams, from the sixth to the seventh of 20.0 grams, and from the seventh to the ninth of 73.0 grams. The composition of the ash of fetuses by Hugounenec and Michel, of the new born by Camerer (27) and Söldner (206), and the premature baby by Langstein and Edelstein (121) differ so little that they fall between the limits of analytical errors. The mineral analyses of Camerer and Söldner are given in table 3, and are also calculated as normal solutions. The total ash value of the fetus is 11,970 cc. 0.1N of acid radicals, 18,680 cc. of basic radicals, a result of 6710 cc. of base. The mother must, therefore, supply to the fetus in basic radicals from the fourth to the fifth month

186 cc., from the fifth to the sixth month 1000 cc., from the sixth to the seventh month 3736 cc., and in the last two months 13,600 cc. Stated in other terms, for the last two months the mother must provide daily 230 cc. of basic radicals, 145 cc. 0.1N of acid radicals, resulting in 85 cc. of base. For practical purposes it is sufficient to consider that for the last one hundred days of pregnancy the mother must supply to the fetus salts in the proportions shown in the table, amounting to 1.0 gram of ash per day. This does not take into account the needs for the placenta or the uterus and hence should be considered the minimum of additional salts required for the fetus alone. The value of base removed for the fetus, 85 cc. 0.1N per day, is sufficient to be

TABLE 3  
*Retention of mineral salts by fetus*  
Per gram of ash

	GM.	CC. 0.1 N		GM.	CC. 0.1 N
Ca.....	0.279	140.0	P.....	0.164	96.0
Mg.....	0.0086	7.2	S.....	0.008	5.0
Na.....	0.057	24.8	Cl.....	0.0661	18.7
K.....	0.058	14.8			
			Acids (2).....		+119.7
Bases (1).....		-186.8			
Acids (2).....		+119.7			
Balance (3) = (1) - (2)...		-67.1			

considered a cause for the acidosis shown by Hasselbach to exist in late pregnancy. As a minimum, therefore, the diet of the woman during the last three months of pregnancy should contain an addition of 150 cc. 0.1 N base per day.

*Infant.* The fetus was shown to retain considerable amounts of minerals. So, too, must the infant show retention of minerals during its growth. In a study of its metabolism, intake and output must also be considered. As a result of the interest aroused by the Breslau School, numerous papers have appeared, reviews of which are found in Meyer and Schloss. But in one particular or another they are incomplete and therefore unsuited to the present discussion. Only five cases on normal infants contain data sufficiently complete to permit discussion of the acid-base relationship—of these, two are cases on breast feeding and three on artificial feeding. Blauberg (20), (21) was the first to study the complete mineral metabolism. He

studied two babies, one breast fed and one artificially fed—during the same period in which Rubner and Heubner (176) studied the latter's energy metabolism. Therefore, the data are especially valuable since they permit correlation with other aspects of metabolism.

*Breast feeding.* The breast-fed baby studied by Blauberg (21) was four and three-quarter months old, weighed 6740 grams and gained 10 grams daily for the six days observed. The values of the minerals calculated in normal solutions, table 4, show a retention of 8 cc. of base per kgm. per day.

TABLE 4  
*Base balance of infants*

Type of feeding.....	Breast fed	Breast fed	Cow's milk	Cow's milk	Cow's milk
Author.....	Blauberg	Tobler and Noll	Blauberg	Shohl and Sato	Shohl and Sato
	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N
Feces value { (1) bases.....	-54	-70	-545	-477	-332
(2) acids.....	+14	+18	+261	+123	+ 90
(1) - (2) = (3) total.....	-40	-52	-284	-354	-242
(4) bases.....	-66	-49	-376	-357	-289
Urine value (5) acids.....	+40	+33	+368	+570	+366
(4) - (5) = (6) total.....	-26	-16	- 8	+213	+ 77
Total output (7) = (6) + (3).....	- 66	- 68	-292	-141	-165
Food value (8).....	-118	-147	-613	-255	-211
Positive base balance (9) = (8) - (7)....	- 52	- 79	-321*	-114	- 46
Positive base balance per kilo.....	8	18.2	26	12	8

\*When 199 cc. 0.1 N are subtracted for negative balances, the value is -222 0.1 N.

Similarly, the baby studied by Tobler and Noll (224) was two and one-half months old, weighed 4 kgm. and gained 25 grams daily for the six days observed. The positive base balance amounted to 18.2 cc. 0.1 N. This is larger than for the baby studied by Blauberg, but the gain in weight was proportionally greater. Also, even though the baby was smaller, he took more food.

*Artificially-fed.* In Blauberg's second study (20) the baby was seven and one-half months old, weighed 7570 grams, gained daily 21.66 grams for the six days observed, and was fed cow's milk and added lactose. Unfortunately for the value of this experiment, there were

negative balances of chlorine, sulphur and sodium. The baby should show gains for all the minerals. We have in table 4 made a correction for the negative balances, but have not estimated the probable retentions. The value of 26 cc. retention per kilo would then probably more nearly equal the balances of the following two cases.

In the two cases of Shohl and Sato (205), the babies were seven and one-half and nine months old, weighed 8.7 kgm. and 5.5 kgm., gained daily 15 grams each for the three days observed. They were fed on cow's milk and cane sugar. They showed a positive base balance of 12 cc. and 8 cc. 0.1N. Therefore, the metabolism in infancy results in a base retention per kilo per day of 10 to 15 cc. 0.1N.

*Child.* The data for infant metabolism are voluminous—that for children is rare. Herbst's excellent study (84) is lacking only in the sulphur balance. Schwarz has supplied data on the sulphur metabolism and its relation to the nitrogen so that these values may, for orientation purposes, be substituted. However, we prefer to consider the only complete mineral metabolism study of children available, that of Sawyer, Bauman and Stevens (184). They have studied the metabolism of two children, ages five and eight years, weighing 22.5 and 23.0 kgm., for three-day periods.

We have recalculated their data, not considering ammonia as an alkali, and also calculated the value of the food which they analyzed. Their figures, as shown in table 5, resolve into a retention of 10.0 cc. and 14.5 cc. 0.1N base per kilo per day. The values are practically the same as those found for infants.

The value of retention can be evaluated, as for the fetus, from the composition of the ash of the body. Such figures for normals are more difficult to obtain in children and adults and more data are urgently needed. Brubacher was unable to obtain complete autopsies. The outstanding conclusion from analyses (36), (207), (216) is that the composition of the body of a child, in respect to its minerals, is very similar to that of the fetus and new-born, and is constant in health and disease. As in the fetus, the ash increases at a more rapid rate than does the total weight. If we calculate the ash as 3.3 per cent of the weight at birth and 4 per cent at the end of the first year and the weight at birth as 3.3 kgm. and at the end of the first year as 10.0 kgm., then the ash increases from 100 grams to 400 grams. The total alkali value of the body as calculated from table 2 is  $400 \times 67.1$ , or 26,840 cc. 0.1N. This represents the total alkali store of the body at one year. The total increase from birth is 20,100 cc. 0.1N, which equals 58 cc. per day.

Dividing this by the average weight of 6.6 kgm., there is found to be a retention of 9 cc. 0.1N base per kilo per day. This value, obtained by calculation, shows close agreement with the value from metabolism studies of 10 to 15 cc. 0.1N cc. per kilo per day.

*Distribution of retained base.* What becomes of the base which is stored? Is it possible to account for a major portion? Obviously, as the body grows its content of alkali increases. Among the substances which might neutralize base the first which will be considered is body protein. If a baby weighs 3300 grams at birth and triples its weight in a year, it grows at the rate of about 20 grams a day. If we assume 15 per cent of the body weight as protein, then it gains 3 grams of protein a day. If the molecular weight of protein is 15,000 and it is considered a monobasic salt, then to form an alkali protein salt would require a base  $\frac{3}{15,000}$  liters normal, or 2 cc. 0.1N alkali per day.

If we calculate the "alkaline reserve" in the manner of Palmer and Van Slyke, assuming the body is 70 per cent fluid and this is 0.03 N  $\text{NaHCO}_3$ , then the baby grows  $\frac{6600 \times 0.03 \times 0.70}{365}$  equals 4 cc. 0.1N alkali per day.

The value of the bones must be taken into account since they are known to be largely alkaline phosphate and carbonate, since in infancy the largest deposition of bone growth takes place, and since the bone becomes increasingly rich in carbonate with advancing age. The bone ash at birth can be taken as approximately five-sixths of 100 grams, or 83 grams, and at one year as five sixths of 400 grams, or 333 grams, representing a daily gain of approximately 0.7 gram of ash. If we assume that the ash is 20 per cent  $\text{CaCO}_3$  and 80 per cent  $\text{Ca}_3(\text{PO}_4)_2$ , each gram of  $\text{CaCO}_3$  would require 200 cc. base and each gram of  $\text{Ca}_3(\text{PO}_4)_2$  40 cc. base. Therefore,  $0.80 \times 40$  equals 32, and  $0.20 \times 200$  equals 40, and for each gram of bone we have 72 cc., or 50 cc. of alkali for bone growth for one day. Thus we have accounted for 2 cc. protein, 4 cc. alkaline reserve, 50 cc. bone, alkaline requirement 56 cc. per day. This leaves only 2 cc. of alkali to be accounted for, as the value of 58 cc. represents the total requirement.

*Fats.* Fat in excessive amounts, or in ordinary amounts in certain pathological states, has been shown to produce so-called soap stools in infants and children. Keller (108) demonstrated an increase in ammonia in the urines of such infants. Steinitz (216) showed under similar conditions a negative balance of sodium and potassium. The

excretion of these radicals diminished slightly in the urine, but increased greatly in the stool. He therefore suggested that elimination of soaps caused a loss of bases, which left the body richer in acids. This he called a relative acidosis. Rothberg (174), for calcium, and Birk (12), for magnesium, were able to show that in chronic nutritional disturbances increased fat caused negative balances. Keller (109), Freund (56), Bahrdt (7) and Givens (65) corroborated these findings. Thus very strong evidence has been collected to establish the theory of relative acidosis.

Sawyer, Bauman and Stevens have also studied the effect of fat on mineral metabolism. On the same two children as were studied during normal periods, they determined the balance of all the minerals when fat was substituted in their diet for an equal number of calories of carbohydrate. Acidosis was produced. The alkali reserve which was 50 and 49 vol. per cent on the normal diets was reduced to 29 and 37 vol. per cent. The values in table 5 show that the positive base balance was reduced in C.G. from 10.0 cc. (normal diet) to a negative balance of 0.8 cc. 0.1N (high fat diet) per kilo per day, and in R.W. from 14.5 cc. (normal diet) to 6.0 cc. 0.1N (high fat diet) per kilo per day. During the periods when the children were in a state of acidosis the alkali retention was reduced to half the normal or eliminated.

The justification of Steinitz's theory of the action of fat is not tenable upon the finding of negative balances. The acidosis is only in part caused by loss of bases in the feces. It depends also upon the failure of the urine to secrete acid. Ammonia excretion decreased in one case of Sawyer, Bauman and Stevens. Therefore, ammonia is not an essential feature of the acidosis, but only occurs when organic acids are also excreted in the urine.

The mechanism by which the acidosis developed can be seen by studying the paths of excretion, table 5. The food value for three days for the normal periods contained 363 cc. less acid than in the fat periods. The value of the urine for C.G. was +1459 cc. and +1253 cc., and R.W. +1787 cc. and +1698 cc. The value of the stools for C.G. was -343 and -520 and for R.W. -349 and -478. The value of the total excretion for C.G. was +1116 and +733 and for R.W. +1438 and +1220. The acid excretion in the urine was actually reduced in both cases by fat, 206 cc. for C.G. and 99 cc. for R.W. The value of the stools was alkaline and was increased in both cases by fat, 179 cc. for C.G. and 129 cc. for R.W. Therefore, the acid

excretion was diminished both by a less acid urine and a more alkaline stool. The balance is a result of three coöperative factors: increased acid in the food and diminished acid excretion in the urine and feces.

*Adults.* The only complete study of the mineral metabolism of the adult has been made by v. Wendt (234). He has determined the mineral balance of two subjects—G. and L.—on a normal diet, with varying salt content, for four days. On two days the data are complete. Unfortunately, in the data he did not give the values of sodium

TABLE 5  
*Sawyer, Bauman and Stevens—Base balance of children*

	C.G., NORMAL (cc. 0.1 N)	C.G., HIGH FAT (cc. 0.1 N)	R.W., NORMAL (cc. 0.1 N)	R.W., HIGH FAT (cc. 0.1 N)
Feces value { (1) Bases.....	-905	-1366	-1149	-1135
(2) Acid.....	+562	+ 846	+ 800	+ 657
(Difference 1-2) (3) Total.....	-343	- 520	- 349	- 478
Urine value { (4) Bases.....	-3738	-4482	-3446	-4326
(5) Acid.....	+5197	+5735	+5233	+6024
(Difference 5-4) (6) Total.....	+1459	+1253	+1787	+1698
Total output (difference 6-4) (7)....	+1116	+733	+1438	+1220
Food value (difference 7-8 (8).....	+ 426	+789	+ 426	+ 789
Base balance for 3 days.....	pos. 690	neg. 56	pos. 1012	pos. 431
Base balance for 1 day.....	pos. 230	neg. 17	pos. 334	pos. 134
Base balance per kilo per day.....	pos. 10.0	neg. 0.8	pos. 14.5	pos. 6.0

and potassium separately and the values assigned to them are approximated by calculating the average ratio of Na/K in the food as 3/1. The data would be more valuable if the subjects were in equilibrium throughout the experiment and studied for longer periods. The food amounts to -27 cc. 0.1N. There are positive base balances for G. of 973 cc. 0.1N and 226 cc. 0.1N. For subject L., similarly, there are positive base balances of 759 cc. 0.1N and 240 cc. 0.1N. per kilo per day; this equals a retention of 13.7 and 3.2 cc. 0.1N for G. and 13.3 and 4.2 cc. 0.1N for L.

*Pregnancy and lactation.* The requirements of minerals for the adult man may be very different from those of a woman during pregnancy

and lactation. In order to retain her own store of minerals, she must store a surplus for the fetus. No complete studies in mineral metabolism are available for an estimation of the balances in humans. Therefore, Forbes' studies (48) on the metabolism of the milch cow are offered as suggestive. Forbes, with others, has published complete mineral metabolism studies on forty-nine cows, for twenty day periods, throughout the yearly cycle of pregnancy and lactation. Minerals have been added, including various forms of calcium and phosphorus. The data are in such form as to permit calculation of the base balance, as well as the balances of the individual constituents. His results show negative balances, regardless of the mineral content of the diet, during lactation, and lactation combined with pregnancy. Not until lactation has ceased and late in the next pregnancy does the balance of calcium and the other elements become positive.

TABLE 6  
*Forbes' data on metabolism of the milch cow*

WEIGHT	DAILY GAIN	PERIOD	COW	SUPPLE	PERIOD OF LACTATION	PERIOD OF GESTATION	MILK	BASE BALANCE PER KGM. CC. 0.1 N
<i>kg.</i>								
519	7.08	II	3		1-7		47	pos. 2.4
514	1.11	II	10		nearly dry	178-193	10	pos. 5.7
579	0.17	I	4		dry	240-259		neg. 7.0
594	0.50	II	4	Ca.	dry	275-279		pos. 5.8

Only four experiments from the whole series will be considered for the purpose of illustration, as shown in table 6. In three of the four cases there is a positive base balance per kilo per day, amounting to 2.4, 5.7, and 5.8 cc. 0.1N; in one, there is a negative base balance of 7.0 cc. 0.1N. In cow 3, period II, the base balance is positive because of excretion of acids. In cow 4, period I, the base balance is negative because of retention of acids. In the remaining two cows the base balance is positive due to retention of alkali. A base retention during pregnancy and lactation is the result of two causes: Milk secretion causes a negative base balance, and the alkali requirement of the fetus causes a positive base balance. In the pregnant cow the alkali retention is half that of the normal infant.

Partial studies in the mineral metabolism of pregnant women have been reported by Landsberg (118), (119). He has studied the metabolism of nitrogen, phosphorus, sulphur, magnesium and calcium. He

concludes that from the second to the tenth month of pregnancy the mother on very liberal intakes of 16.0 grams N, 3.0 grams = 1740 cc. 0.1N P, 0.5 gram = 402 cc. 0.1N Mg, and 2.5 grams = 1250 cc. 0.1N Ca, shows positive balances of all these elements. Even after deducting the amount necessary for the fetus—which he calculated from data already cited under the fetus—he found large positive balances for the mother. The fetal adnexa and the growing uterus must require their quota, or the mother must be storing minerals upon which to draw during lactation.

No metabolism experiments upon women during lactation are available. We can, however, compute, from the analysis of breast milk, that quota of alkali which she must supply. In Blauberg's case the value of the breast milk consumed was 188 cc. 0.1N alkali per day; in Tobler and Noll's the milk equalled 147 cc. 0.1N alkali per day. Hence, this amount of minerals must be given as a minimum to the nursing mother in addition to her normal requirement. The practical importance of determining what is an adequate mineral diet and the alkali requirement for a pregnant or nursing woman warrants further investigation.

**ACID-BASE EQUILIBRIUM IN THE BODY.** The acid-base equilibrium of the body is governed by the intake of acids in the food and their excretion in the urine and feces.

*Acid-base value of food.* The word alkali comes from the Arabic and means marine plants. Liebig speaks of the acid ash of meats, meat juice and cereals and the alkali ash of vegetables. The value of foods as sources of an excess of acid or base forming elements was first expressed in terms of normal solutions by Meyerhofer (147) in 1902 for mineral springs; and by Dennstedt and Rumpf (35) in 1904, for animal tissue. To Sherman we really owe our present knowledge of the subjects. He has given (208), (210) tables of the acid or base value of foods in cubic centimeters of normal solutions per 100 grams and per 100 calories. Forbes (51) published from his own experiments and analyses in the literature a table of calculations of acid-base value of foodstuffs. The most complete data are those of Berg.

The significance of acid and alkaline diets has been reviewed by Greenwald (15, p. 413), who states no relation to metabolism has been proved. Blatherwick has found the diets in army camps to average 22 cc. 0.1N acid. Sherman found an average value of 180 cc. 0.1N acid on freely chosen mixed diets. The civilian diets should approximate the requirements but the army diets should be more satisfactory

because every effort was made to give the soldiers the best diet available. These studies were made over short periods. Even during long periods the acid or alkaline value may not determine life or death but may lead to diminished efficiency or to pathological conditions. Small variations in the acid base value of the diet probably have little significance provided the total salt content is adequate; for the body can excrete that portion which is not essential. A person or group of persons may take too acid a diet for one period and correct its effects by an alkaline diet at another period: for example, diets acid from meat and cereals in the winter, and diets alkaline from fruit and vegetables in the summer. Groups which select special limited diets, such as nuts, fruit, vegetables, or meat alone would make interesting studies. Acid diets have proven beneficial in infantile tetany and their use has been suggested in rickets. Alkaline diets are essential for infancy where growth is rapid. Their use is strongly indicated in pregnancy and lactation and such pathological conditions as diabetes. From Chittenden's study of athletes and soldiers on vegetable food it appears that on alkaline diets the body is capable of more sustained effort than on acid diets.

*Acid-base value of the urine.* The urine is the most important means of maintaining the acid-base equilibrium of the body. Through the excretion of acid or alkali the organism maintains a constant or nearly constant condition, whether the food is acid or alkaline. This function is so delicate that the excretion varies from day to night or from hour to hour. During secretion of HCl for digestion, the urine is alkaline. Even the effect of forced breathing, which removes the CO<sub>2</sub> from the blood and makes it more alkaline, is immediately reflected in the secretion of an alkaline urine.

Gaethgens and Stadelman (210) were the first to calculate the acid-base value of the urine. They analyzed the minerals and expressed their values as equivalents of sodium. How this excretion is accomplished and how the factors vary with acidity or alkalinity has recently been studied (81), (167), (225). The urine is normally acid. This acidity depends upon, 1, the excretion of acid phosphate; 2, acid which is neutralized by ammonia formation; 3, organic acids; 4, acid neutralized by inorganic minerals; 5, carbonic acid, as bicarbonate and carbonate. Normally the urine contains 500 to 1000 cc. of acid, depending upon the diet. The acid (Palmer) is divided as acid phosphate, 100 to 200 cc., acid neutralized by ammonia, 300 to 400 cc., organic acid, 0 to 150 cc., carbonates, 10 to 40 cc. The free acid represents half the value, and the ammonia, the other half.

The organic acids—uric, hippuric, lactic—are usually small but under varying conditions of diet and exercise they may be 150 to 200. Under pathological conditions they may greatly exceed this value. The phosphoric acid excreted in the urine depends upon the amount in the diet. That portion of the acid excretion which is neutralized by basic minerals is not represented in the values obtained by titration methods, but is only detected when mineral metabolism is investigated. Under normal conditions mineral loss is important. The urine normally excretes nearly all the chloride, 80 per cent of the sodium and potassium and sulphur, 50 per cent of the phosphorus, but only 20 to 30 per cent of the calcium in adults and only 5 to 10 per cent of the calcium in infants. The mineral constituents of the urine are controlled not only by the diet but also by the osmotic, ionic and acid-base equilibria.

*Acid-base value of feces.* That the feces play little or no part in normal acid base equilibrium and that the minerals there found represent merely those unabsorbed has been generally assumed. Even if mineral radicals were not excreted or secreted by the bowel, the factors which govern their absorption would control the acid-base equilibrium in the body. The work of Goldschmidt and Dayton (68), (69) indicates that the bowel does play an important part in the transfer of minerals to and from the blood. Measurements of pH of the contents of the small intestines by McClendon and of the feces by Hawk show such constancy of the reaction as to indicate some regulatory process. Further, Ylppö has shown that the pH of the feces after breast milk feeding is acid—about pH 5.0—and after cow's milk feeding is alkaline—about pH 8.0. Other evidence of the regulatory process is shown by a study of the paths of excretion; for example, nearly all the calcium leaves the body through the feces, and the chlorine through the urine.

A method on the titration value of the stools was proposed by Blau-berg (178). He studied the amount of acid or alkali necessary to neutralize the dried stool. Similar studies have been made by Talbot and Hill (219). The interpretation of these values is difficult. The relations of acid to base are altered by drying. Even if correct values on twenty-four hour metabolism periods were obtained, they would represent only the unbuffered states—equivalent to the free acid excretion in the urine. They would not represent the total acidity. Other methods are needed.

By analysis of the mineral radicals, expressed in terms of sodium equivalents, Fleitmann (cited by (72)) showed that the stools were alkaline. A normal value for adults is 200 to 300 cc., as determined by v. Wendt, and for the infant, 500 cc. 0.1N base per day. Studies by Shohl and Sato show that the bowel, by excretion of alkali, normally participates in regulation of the acid base equilibrium.

**THE EFFECT OF ACID ON METABOLISM.** The first important studies on the effects of ingested acid were made at Dorpat. Under the direction of Buchheim appeared a series of papers on the excretion of mineral acids in the urine. In 1877 a classic paper by Walter (230) showed that acid led to poisoning and death. His experiments on dogs demonstrated: 1, a diminished  $\text{CO}_2$  in the blood, and 2, an increased amount of ammonia in the urine. Salkowski (182) emphasized that especially in herbivora acid caused a loss of alkali minerals in the urine.

Experiments in acid feeding and the resultant condition "acidosis" have developed along three lines:

1. The effect of acid on  $\text{CO}_2$  in the blood and other factors in the acid-base equilibrium in the blood. This is reviewed in a forthcoming article by Wilson (237).

2. The effect of acid on the  $\text{NH}_3$  in the urine. Controversies have arisen over the difference between carnivora and herbivora and the fate of various nitrogenous elements, but the consensus of opinion of nearly a hundred investigators has substantiated Walter's finding—when acids are given, the urinary  $\text{NH}_3$  is increased.

3. The loss of alkali minerals also in the urine of acid feeding has now been well established. When 250 cc. of acid are given, all the acid radicals and also 100 cc. extra bases are excreted in the urine. Stehle and MacCarthy (212) have recently reviewed this question in both dogs and men, as have Givens and Mendel (66), Gamble (62), and Shohl and Sato for infants.

Acid poisoning is produced not only by the mineral acids, hydrochloric, sulphuric and phosphoric, but also by organic acid. Ingested benzoic, lactic or organic acids produced in metabolism have been shown to cause acidosis. Of the latter,  $\beta$  oxybutyric acid in diabetes is quantitatively the most important; as much as 80 grams may be excreted daily. Organic acids are also formed in methyl alcohol poisoning, pneumonia, and in intestinal disorders in infancy.

The mechanism of the action of acids on the urine, calculated on the daily output, shows that, 1, the acidity of the urine increases up to pH 4.6; 2, the amount of free acid excreted—measured by the titra-

table acidity—increases up to 2500 cc. 0.1N; 3, the phosphate is excreted in increased amount, two or threefold, and more is converted to acid phosphate, so the excretion of acid increases up to 700 cc.; 4, the organic acids may amount to 10 liters 0.1N or more. In the acid urine 30 per cent is excreted as free acids, thus does not require base and ammonia for neutralization. 5, The ammonia shows the greatest increase. In diabetes it may be as large as 7000 cc. 0.1N; 6, no accurate data are available for the loss of alkali minerals in extreme acidosis, but, by estimate, it may amount to 500 cc. 0.1N.

That the stools are active in regulation of the acid base equilibrium is becoming more apparent. Shohl and Sato have stated that the stool participates in the regulation of acid base equilibrium and in emergency it may excrete acid. Steinitz and Sawyer, Bauman and Stevens have also shown that the feces may even cause acidosis by excreting alkali.

From the point of view of mineral metabolism, acid causes a diminution in the retention of all the elements, but the effect is greater upon the alkali radicals than the acid radicals. In a baby on a milk diet, when 250 cc. 0.1N HCl (Shohl and Sato) and 750 cc. 0.1N (Gamble) were given, one-seventh of the acid was retained and the rest excreted by the urine. The infant on a diet made neutral by the addition of HCl excretes an excess of acid and retains base. The body further responds to the acids by furnishing alkali from the blood tissues and bones. Acid lowers the alkali reserve through excretion of  $\text{CO}_2$  by the lungs. There is a rearrangement of the equilibria in the blood, causing a diminished pH. Acid ingestion results in a diminished base balance or in a negative base balance.

EFFECT OF ALKALIS ON METABOLISM. Alkalosis is the term used to indicate an increase in the pH of the blood produced by alkalis. Forced breathing, high altitudes and cold baths also have been shown to produce this condition. The effect of alkalis on metabolism of minerals is little known. The only complete metabolism studies which have been published are those of v. Wendt (for a single day) and of Shohl and Sato (for a single period). The latter have emphasized that the bowel can excrete alkalis. Together with the urine, the feces protect the body from alkalosis. The urine becomes alkaline by excreting sodium; the stool by excreting calcium. However, an increased retention of alkalis takes place. This retention affects the water metabolism; a gain in weight or even edema occurs after alkali treatment.

The material on the metabolism of alkalis is not large, though their use in medicine has been extensive since the time of the ancients. A review of the early literature is given by Quenoille (172). Wöhler's researches (239) constitute the beginning of scientific knowledge. He showed that the organic salts of alkalis are changed to carbonates in the body and make the urine alkaline. The early work following Benec Jones' is largely confined to the effect of alkali on the acidity of the urine, or on the nitrogen metabolism. The discovery of  $\beta$  oxybutyric acid by Minkowski (151) in the acid intoxication of diabetes and the recognition by Stadelmann of the increased ammonia in the urine, gave new significance to the effect of alkalis. Stadelmann felt that if an acid intoxication existed, alkalis could be used to neutralize the acids. He therefore carried out, through his students at Dorpat, a series of researches on the effect of alkalis and published, in 1890, (210) a monograph of the work of Burchard, Klemptner, Beckmann, Hagentoren and Kozerski. This work is important and difficult of access. It shows great care in the planning of experiments, in analytical methods, and in the review of the literature.

Burchard determined the urea, uric acid, ammonia and chlorides in the urine, and in some of his experiments the nitrogen in the feces also. On a constant diet he took sodium bicarbonate, increasing from 5.8 grams per day to 27.0 grams per day. The last dose caused a diarrhea and he took small amounts of opium. His conclusions are that alkalis have no dyspeptic action but make the urine definitely alkaline and increase the volume. Taken for a long time, they slightly decrease the nitrogen output; definitely decrease the ammonia output to minimum amounts, they diminish the uric acid. Klemptner repeated and confirmed Burchard's work.

Beckmann took for his part of the study the analysis of sodium, potassium, calcium, magnesium and ammonia. Unfortunately he did not analyze the minerals in the feces. The output of sodium was larger than the intake; in one case the excretion was 150 per cent of the intake. A small loss of potassium and chloride also occurred. The output of calcium and magnesium was not influenced. The ammonia was depressed in proportion to the amount of alkali given. The chlorides excreted are insufficient to account for the excess of sodium, so that it must be excreted in part as carbonate.

Hagentoren also analyzed Beckmann's urine and determined phosphorus, nitrogen, uric acid, total sulphuric acid, neutral sulphur, preformed sulphate, hydrochloric acid. Calculating both acids and

bases including ammonia, as equivalents of sodium, he showed that normal urine contains an excess of base equivalent to 0.49 sodium, but of this the ammonia amounts to 0.58. When alkali is taken, the excess of base is much larger. When sodium citrate is taken, sodium chloride and potassium chloride are withdrawn from the body in proportion to the amount of salt taken. With sodium bicarbonate, chlorine is not removed. The output of sulphur or phosphorus is not affected. Neutral sulphur is increased. Sodium bicarbonate and sodium citrate are in part excreted as carbonates.

Kozerski analyzed sodium and potassium by improved methods, and attempted to discover whether sodium citrate differs from carbonate in its effect upon the urine. He, too, was on a weighed and measured diet, and determined not only the urine but also the nitrogen of the feces. He unfortunately did not determine any of the bases in the feces. Amounts of sodium larger than 7.0 grams increase the output of potassium and chloride. The organism eventually diminishes the excessive output of sodium and chloride. When the alkali is stopped, the urine becomes acid on the second day and the chloride and potassium output become normal. Alkali causes no loss of nitrogen. Sodium carbonate has exactly the same action which Beekmann found for citrate. The output of sodium increases as the intake, so that large doses bring about a loss of sodium from the body.

The effect of alkalis on uric acid has been much discussed in the literature. It led to the practice so much in vogue fifty years ago of giving alkalis, especially lithium salts, to drive excess of uric acid from the body. Lithium urate is the most soluble salt of uric acid but it does not prevent the insoluble forms of the salt from precipitating in joints of patients with gout. Alkaline urine does dissolve uric acid. The amount of uric acid held in solution is a function of the pH.

Phosphates in the urine created a similar problem. Acid urines hold phosphates in solution; alkaline urines do not. The effect upon the elimination of phosphates by the body depends upon the balance. Alkali causes less phosphate to be excreted by the urine and more by the bowel. In infants the total phosphorus excretion is increased by both acids and alkalis. The action of alkalis can be summarized. In the urine 1, the reaction becomes alkaline up to pH. 8.4—which results in a negative value for the titratable acid up to 200 cc. 2, The ammonia is diminished to minimal amounts and may actually be eliminated. This increases alkali excretion up to 500 cc. 0.1N. 3, The phosphate is converted to alkaline phosphate, an increase of alkali

excretion up to 400 cc. 0.1N. 4, The organic acids, if present, are completely neutralized. 5, The carbonates may be present up to 1000 cc. for neutralization of base. 6, The chlorine and potassium are slightly increased. The alkali excretion is accomplished by means the opposite of those in acid excretion. In acid excretion the pH is diminished and the acidity regulated by the phosphates. Ammonia is increased to neutralize the acid. Carbonate is negligible. In alkali excretion the pH is increased and the acidity regulated by the carbonates. Ammonia is negligible. Carbonate is formed to neutralize the base.

In the feces there is an increased excretion of alkali, mainly as soaps, phosphates and carbonates of calcium. This together with the urine removes excess alkali.

In the body alkali retention is increased, which causes an increase in body fluids and also makes the blood more alkaline.

CONCLUSIONS. What is the significance of the requirement of minerals in health? They furnish no energy. They are not consumed. Why does the body waste them? Is there any significance in the amount stated as a requirement? If the diet were altered the requirement might also be altered. Rubner (cited by Greenwald) states that the Japanese diet is adequate, although low in calcium, because it is also low in fat. If it were low in phosphate, could it not also be low in calcium? In other words, a relationship exists between each mineral element in the diet to the total caloric intake, to the organic factors, to each of the other mineral constituents and to the acid-base balance of the body.

In the physiologic literature of the last twenty-five years the relationship of one element to another has been made clear, especially its significance for the mechanism of the heart beat, of nerve stimulation, etc. Quite recently Loeb has shown the new significance of the ionic relations in physiology, according to the Donnan equilibrium.

Not only as regards amounts are minerals of great significance in relation to one another, but also as regards concentration. Their concentration determines the osmotic pressure relationships of the body and hence the water metabolism. In order to solve the problem in any real sense, to establish the requirements of any one element, all others must be held constant while that one is varied which gives a number of permutations.

In growth and pregnancy and in cases of neoplasms, minerals must be retained. In adult life an equilibrium should exist. Even under

the most favorable conditions a loss from the body and replacement by food continually occurs. No satisfactory explanation for the mineral flux has ever been offered. A possible approach to the problem may be an understanding of the significance of the "alkaline tide" observed by Bence Jones.

The minerals, therefore, aid in maintaining at least three kinds of equilibria: 1, osmotic, 2, ionic, and 3, acid-base. A normal standard of base balance in the human body has been defined. The effect upon the base balance has been determined for alkalosis and acidosis.

#### SUMMARY

1. Mineral metabolism can be studied simply and effectively in terms of normal solutions. Measurement of the acid-base balance is thus made possible.

2. The normal mineral requirements per man per day approximate 150 cc. 0.1N acid.

3. The fetus shows a retention of alkali for the last one hundred days of gestation equal to 67.0 cc. 0.1N alkali per day.

4. The infant and child show a positive base balance amounting to 10 cc. 0.1N base per kilo per day.

5. The adult balance approximates equilibrium.

6. Pregnancy and lactation require additional alkali—a minimum of 150 cc. 0.1N base per day.

7. Acid diminishes a positive balance or causes a negative base balance.

8. Alkali increases the positive base balance.

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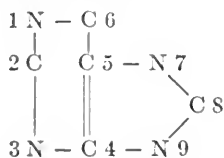
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# PURINE METABOLISM<sup>1</sup>

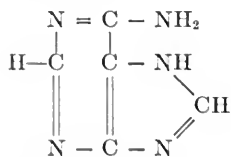
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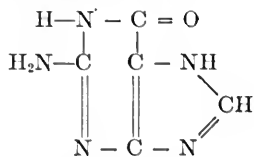
1. PURINES OF PHYSIOLOGICAL INTEREST. Of the many theoretically possible purines, the student of metabolism is chiefly concerned with five, namely, adenine, guanine, hypoxanthine, xanthine and uric acid. As shown by Emil Fischer (74), purines are all derivatives of a common "nucleus" or ring. The structural formulas for this ring, and for its five physiologically interesting derivatives, are indicated below.



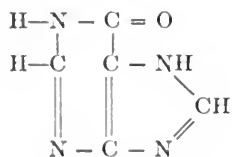
Purine "Nucleus"



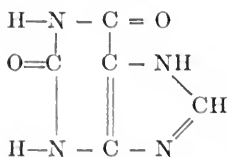
Adenine (6-amino-purine)



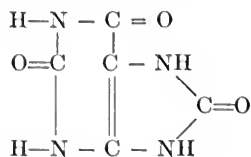
Guanine (2-amino-6-oxy-purine)



Hypoxanthine (6-oxy-purine)



Xanthine (2-6-dioxy-purine)



Uric acid (2-6-8-trioxy-purine)

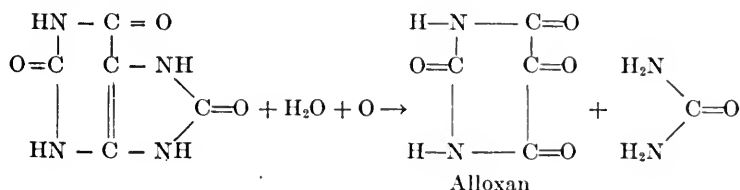
In addition to the above, certain methylated purines enter into the human dietary as ingredients of beverages. Their physiological fate will be referred to later.

Our knowledge of purine chemistry dates back to 1776, when uric acid was discovered by Scheele (267) as an ingredient of human urine, and almost simultaneously but independently by Bergmann (37) as a component of bladder stones. The other purines, with the exception

<sup>1</sup> It has been impossible in the space available for this review to refer to all of the publications related to purine metabolism. We have, therefore, confined our attention to those papers which in our opinion are most important, and which have served to introduce new ideas into the present-day conception of the subject.

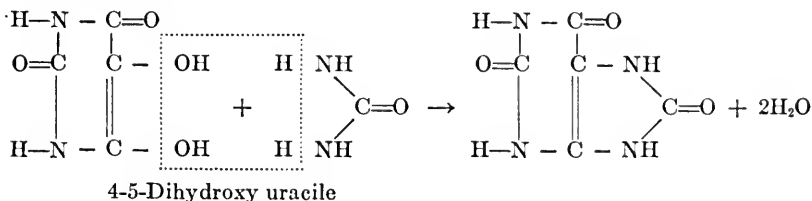
of adenine, were discovered during the first half of the nineteenth century. Marcet (213) separated xanthine from urinary calculi. Guanine, first isolated from guano by Unger (310), was later reported by Virchow (311), (312) as an ingredient of concretions in swine. Hypoxanthine was discovered in heart and spleen tissue by Scherer (268), and later in the muscles of man and the lower animals by Strecker (289). Adenine, the last of the physiologically important purines, was not known until 1885, when Kossel (157), (158), (159), (160) succeeded in isolating it from pancreatic tissue. It remained for Emil Fischer (70), (75), (71), to show the relationship of adenine, guanine, hypoxanthine and xanthine to each other and to uric acid, and to devise methods for their synthesis (72), (73). It is beyond the scope of this article to present in detail the evidence upon which the present conception of the structure of the purines is based. It is sufficient to state that their structural relationship to uric acid has been abundantly established, and that the essential correctness of the uric acid formula is affirmed by the following facts:

1. By the oxidation of uric acid with  $\text{HNO}_3$  Liebig and Wöhler (204) obtained alloxan and urea.



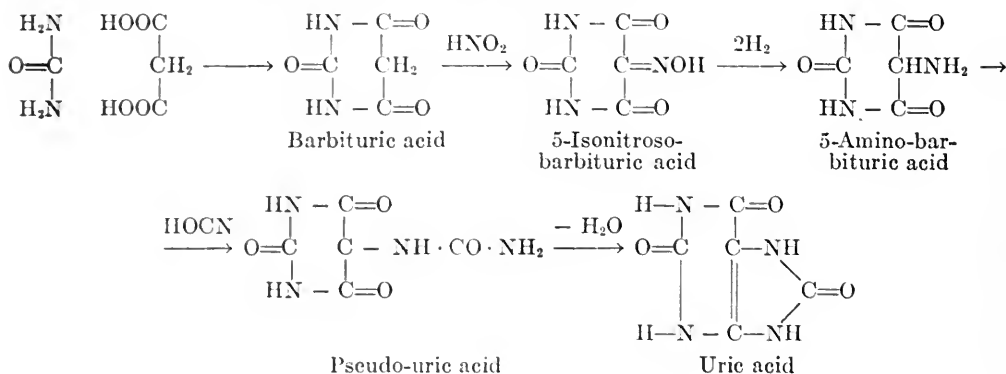
This observation, together with a study of other oxidative disintegrations of uric acid, led Medicus (218) to suggest its correct formula.

2. In 1888 Behrend and Roosen (29) accomplished the synthesis of uric acid by warming a mixture of 4-5-dihydroxy uracile and urea with concentrated  $\text{H}_2\text{SO}_4$ .



3. In 1897 Fischer (72) showed that pseudo-uric acid, previously prepared from malonic acid by Baeyer (20), (21), is readily transformed

into uric acid by boiling with dilute mineral acids. This provided not only a complete synthesis of uric acid from malonic acid and urea, but removed the last vestige of doubt as to uric acid structure.



For detailed evidence concerning the structure of the other purines the reader is referred to the masterly studies of Emil Fischer (74).

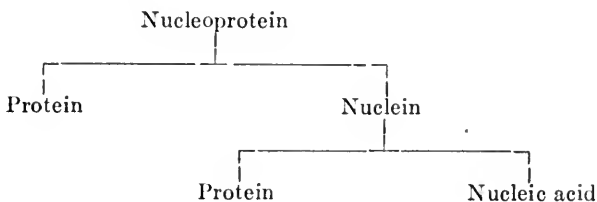
The discovery of the purines as ingredients of physiological materials immediately challenged the attention of both biochemical and clinical investigators. Indeed, attempts to study the distribution of the purines, and to determine their functional relationship to the life processes are among the earliest metabolic investigations on record. Progress soon showed that purines are associated with the nuclei rather than with the cytoplasm of cells, and this in turn led ultimately to the discovery of nucleic acids. It is not our purpose in an article of this scope to enter into a detailed discussion of the chemistry of nucleic acids. Such a discussion would be entirely superfluous in view of the availability of the excellent review of the subject by Jones (130). But inasmuch as the progress made in recent years in the study of purine metabolism has in large measure been the outgrowth of the discovery of nucleic acids, it is necessary for us to summarize in the following paragraphs the *essential features* of nucleic acid structure.

2. THE CHEMISTRY OF NUCLEIC ACIDS. Our knowledge of the chemistry of nucleic acids begins with the work of Friedrich Miescher (227) in 1871. This author, in studying the chemistry of pus cells, isolated a substance soluble in dilute alkali and precipitable with acetic acid, to which he gave the name of nuclein. Nuclein was found to contain phosphoric acid, and to respond to the protein color tests. Later Miescher (228) isolated a similar substance from the spermatie fluid of the salmon.

Nucleins were soon found to be characteristic of all cell nuclei. Hoppe-Seyler (109) isolated a similar product from yeast, and Kossel (154) another from the erythrocytes of birds. In his work upon the nuclein of salmon, Miescher discovered that the protein portion could be completely removed, leaving a protein-free acid. A similar acid prepared from yeast nuclein was later named "nucleic acid" by Altmann (13). In 1894 Kossel and Neumann (163) devised a method for the preparation of the nucleic acid of the thymus gland, and Neumann (243) showed that such acids can be isolated from a large number of nuclear materials.

Miescher did not recognize the relationship of nucleic acid to purines, or that the latter are important ingredients of the former. It remained for Kossel to prove that on hydrolysis nucleins liberate "alloxuric bases" or purines. He detected successively hypoxanthine (152), xanthine (153), guanine (155), (156), and finally adenine (159), (160). The oxy-purines are now known to arise only as secondary products, and not to occur preformed in the nucleic acids. The discovery of the purines immediately suggested an origin for urinary uric acid, which before this time had been regarded as a product of ordinary protein metabolism.

Nucleic acids occur in the nuclei in combination with protein, probably a salt-like union, in which the protein plays the part of a base and is present in excess. These combinations constitute what are known as nucleo-proteins (the  $\alpha$ -nucleoproteins of Hammarsten (101)). Some writers, notably Kossel and Lilienfeld (206), (205), are inclined to the belief that each molecule of nucleic acid is in combination with two molecules of protein, one of which is more readily removed by hydrolysis than is the other. According to this conception, nucleoproteins have the following structure:



On the other hand, Jones is of the opinion that the proportion of protein depends largely upon the method of preparation of the nucleoprotein, and that the "terms nucleoprotein, nuclein and nucleic acid express a relation which means little more than that conveyed by the terms basic lead acetate, lead acetate and acetic acid" (130).

The study of nucleic acids has resulted in an accumulation of evidence indicating that there are only two such compounds in nature, one in the animal cell and typified by thymus nucleic acid, and the other in the plant cell and illustrated by yeast nucleic acid. Despite the fact that numerous tissues of both animal and plant origin have been analyzed, only nucleic acids of these two kinds have been discovered. They differ from each other in their ultimate hydrolytic products as illustrated below.

*Products of hydrolysis of nucleic acids*

<i>Of animal origin</i>	<i>Of plant origin</i>
Phosphoric acid	Phosphoric acid
Adenine	Adenine
Guanine	Guanine
Cytosine	Cytosine
Thymine	Uracile
Levulinic and formic acids	Pentose

Plant nucleic acids are characterized by containing the pyrimidine uracile, discovered by Ascoli (17), and of a pentose. The presence of the latter was proven by Kossel (161). It was identified by Levene and Jacobs (181), (183), (184), (188), (190) as d-ribose, a

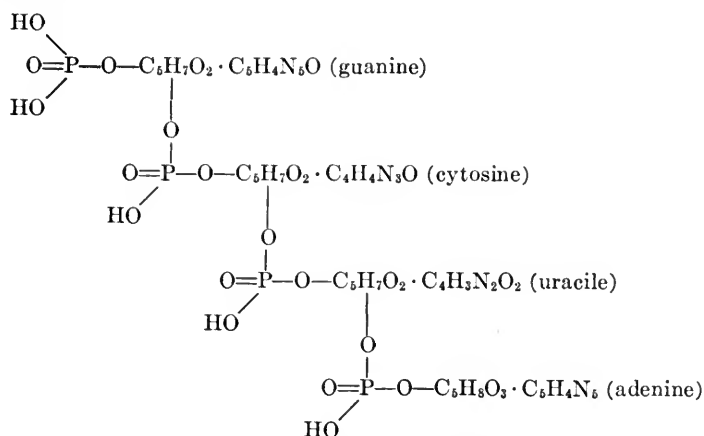


levo-rotatory aldopentose unknown in nature until the work of these authors. Nucleic acids of animal origin contain thymine (cf. Kossel and Neumann (162)) instead of uracile, and an hexose as indicated by the production of levulinic and formic acids (163), (16), (121) on hydrolysis. The nature of the hexose is unknown. On oxidizing thymus nucleic acid with nitric acid, Stendel (284), (286), (287) obtained episaccharic acid ( $\text{C}_6\text{H}_{10}\text{O}_8$ ). More recently Feulgen (62), (63) has suggested that the carbohydrate group may be glucal ( $\text{C}_6\text{H}_{10}\text{O}_4$ ) instead of an hexose.

We are chiefly indebted to Levene and Jones for the information which we have concerning the union of the various hydrolytic products to form the complex nucleic acids. Levene and Jacobs (186), (187),

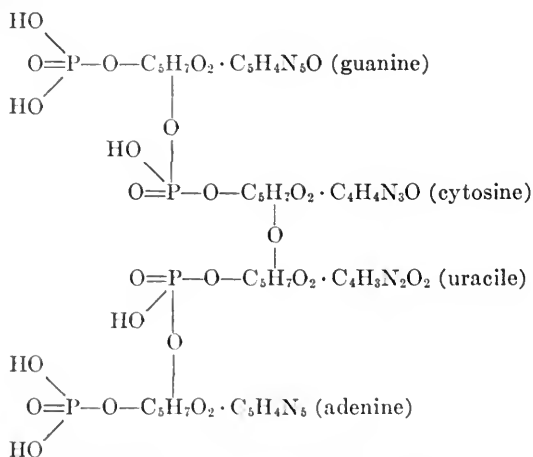
(189) found that neutral hydrolysis of yeast nucleic acid at 175°C. under pressure led to the separation of four crystalline compounds, each composed of a purine or pyrimidine molecule in combination with a molecule of d-ribose. The fact that these substances do not reduce alkaline copper solutions indicates that, at least in the case of the readily hydrolyzable purine compounds, the sugar and base are probably united by a glucoside binding. They are known collectively as nucleosides (195), and are named adenosine, guanosine, cytidine, and uridine respectively, according to the base which they contain. Furthermore, in the nucleic acid each nucleoside is in combination with a molecule of phosphoric acid to form a so-called nucleotide (195), or mononucleotide. The union is between the acid and the sugar (195), and is probably an ester linkage between the phosphoric acid and the primary alcohol group of the sugar molecule. All four of the theoretically possible mononucleotides of yeast nucleic acid are now known (cf. Jones and Kennedy (135), Thannhauser and Dorfmueller (300), Levene (177), (178), (179)).

The facts outlined above led Levene (175) to believe that yeast nucleic acid is a tetra-nucleotide composed of the four mono-nucleotides of adenine, guanine, cytosine and uracile. He (176) has recently suggested the following formula in which the nucleotides are represented as united to each other by ester linkages. Levene (180) is also of the opinion that thymus nucleic acid has an analogous structure, in which the four hexose nucleotides are combined through the phosphoric acid and sugar molecules.



*Levene's formula for Yeast Nucleic Acid*

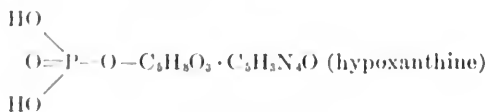
Until recently Jones (131) has advocated an ether linkage between the sugar molecules of the nucleotides. This idea has been accepted by Thannhauser and Sachs (301). Within the past few months Jones (137) has modified his views, and now represents the structure of yeast nucleic acid as follows:



*Jones and Perkins' formula for Yeast Nucleic Acid*

As to evidence for and against each of these formulas, the reader is referred to the original articles.

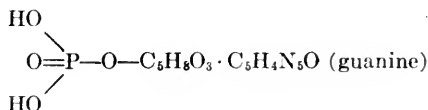
In addition to the true nucleic acids, two compounds of analogous structure have been separated from certain tissues. In 1847 Liebig (203) isolated from meat extract a compound which he named inosinic acid. The structure of this substance has been established largely through the work of Bauer (26), Neuberg and Brahn (242), Hauser and Wenzel (99), and Levene and Jacobs (182), (183), and has been shown to be a mono-nucleotide of phosphoric acid, d-ribose, and hypoxanthine having the following formula:



*Inosinic Acid*

The second substance of this kind is also a mononucleotide. In 1894 Hammarsten (101) prepared two nucleoproteins from pancreatic tissue, from one of which (his so-called  $\beta$ -nucleoprotein) he separated guanylic

acid by hydrolysis. Bang (23), (24), Steudel (285), and Levene and Jacobs (185), (192) have shown that guanylic acid has the following structure:



*Guanylic Acid*

Guanylic acid has also been isolated from a variety of glandular tissues other than the pancreas (cf. Jones and Rowntree (139), and Levene and Mandel (194)). Inosinic and guanylic acids are of peculiar interest in that they contain the sugar (d-ribose) characteristic of plant nucleic acids, and therefore cannot have their origin in the tetra-nucleotide of animal cells. They doubtless account for the wide distribution of pentose in animal tissues. There is no evidence to indicate that they occur in-cell nuclei, but on the contrary, they probably exist in combination with the protein of the cytoplasm.

The discovery and identification of nucleic acids, and of the extra-nuclear inosinic and guanylic acids, served to greatly stimulate investigation of the physiological history of uric acid, the study of which had formerly been pursued in a more or less haphazard manner.

3. THE DIGESTION OF EXOGENOUS NUCLEIC ACIDS (NUCLEOPROTEINS). Abundant evidence has been accumulated indicating that the protein portions of ingested nucleoproteins are split off by the gastric and pancreatic juices with the liberation of free nucleic acids. In 1892 Lilienfeld (205) found that nucleoproteins are converted into proteins and "nuclein" by the gastric secretion, and that the nuclein is subsequently disintegrated by the pancreatic juice into protein and free nucleic acid. On the basis of these observations, and at the suggestion of Kossel, he proposed the scheme of nucleoprotein structure already alluded to (see p. 547). The fact that the proteolytic enzymes of the gastric and pancreatic secretions are capable of removing the protein, and digesting it just as they do other proteins, has been verified by Popoff (252), Umber (307), Harding and MacLean (103), and others. Apparently trypsin is far more effective in this regard than is pepsin.

In this connection the work of Schmidt (274) should be mentioned. Schmidt believed that cell nuclei are digested by the pancreatic juice only, and suggested that the microscopic examination of the stools for cell nuclei following the feeding of nuclear material might be employed as a diagnostic test for inefficiency in pancreatic digestion. Sev-

eral investigators, notably Strauch (288), were ardent defenders of the alleged value of the "Schmidt test." It is likely that both Schmidt and Strauch were deceived by the slowness of peptic digestion under the conditions of their experiments. Both in the *in vitro* experiments and in the clinical diagnostic tests, they used strips of muscle tissue which had previously been hardened in alcohol as the substrate for the action of the enzymes. It is likely that their negative tests are attributable to the relative indigestibility of the alcohol-hardened tissue. That pepsin can slowly digest cell nuclei, even after hardening of the tissue, has been shown by Hesse (107) and Kashiwado (141). The results of the latter investigators, and of van Westenrijk (323), indicate that the Schmidt test is unreliable. Certainly there seems to be no occasion for doubting that the alimentary enzymes, under ordinary conditions, completely digest the protein portion of the nucleoprotein.

The gastric and pancreatic juices are generally believed to be entirely without influence upon nucleic acids. Milroy (230) believed that trypsin slowly removes phosphoric acid in some organic form from nucleoproteins, and Abderhalden and Kashiwado (4) report that part of the phosphoric acid is split off under the influence of the gastric and pancreatic secretions. That such reactions could occur to an appreciable extent seems quite unlikely in view of the investigations of others. Abderhalden and Schittenhelm (5) had previously reported that pancreatic juice collected from fistula animals, either with or without the addition of enterokinase, decomposes thymus nucleic acid in such a way as to destroy its power of gelatinizing, but without the liberation of free purines. They also stated that gastric juice was without effect upon nucleic acid. Levene and Medigreceanu (197) were unable to detect an enzyme capable of decomposing nucleic acids in either the gastric or pancreatic secretions. Furthermore, the early experiments of Milroy are not convincing. This author employed extracts of the pancreas for his experiments, instead of digestive juices collected from fistula animals. It is conceivable that such enzyme preparations may have contained intracellular purine ferments. As we shall see later, intracellular enzymes produce more profound disintegrative changes in the nucleic acid molecule than do alimentary secretions. For the most part Milroy's experiments were made with artificial nucleins prepared by bringing together faintly acid solutions of proteins and nucleic acid. Jones (127) has shown that trypsin is without effect upon the nucleic acid part of nucleoproteins when the latter are purified, and dried by washing with alcohol and ether. Under these conditions there is no liberation of

phosphoric acid. If, however, the nucleoprotein is not so purified and dried, intracellular enzymes from the tissue used in the preparation of the substrate apparently are adsorbed by the nucleoprotein, in spite of frequent reprecipitations, and through their action may lead to erroneous conclusions. Jones' work clearly shows the complete independence of trypsin and the enzyme responsible for nucleic acid disintegration. Other investigators (cf. Biondi (40), Iwanoff (122) and Sachs (265)) also recognized the latter fact, but the experimental data upon which their opinions were based are not as convincing as those of Jones.

Nakayama (240) found an increase in free phosphoric acid when extracts of intestinal mucosa were permitted to act upon nucleic acid. He made the rather remarkable deduction that the enzyme responsible for the splitting of nucleic acid is identical with erepsin. More likely, as pointed out by Jones (130, p. 60), the experiments of Nakayama merely show the similarity in distribution of the two enzymes, and indicate that it is difficult to obtain an erepsin preparation devoid of the enzyme concerned in nucleic acid disintegration. It is quite unlikely that a member of a group of substances which are characterized by such remarkable specificity in action as are enzymes should act upon two substrates so widely different as peptones and nucleic acids.

The most careful and complete experiments reported concerning the action of alimentary enzymes upon nucleic acids are those of Levene and his associates. Using extracts of the intestinal mucosa and pure gastric, pancreatic and intestinal juices obtained from Pawlow fistula animals, Levene and Medigreceanu (197), (198) found by optical methods of study that three groups of enzymes are concerned in nucleic acid digestion, instead of a single ferment, "nuclease" (122), as had formerly been believed. They employed as substrates not only yeast and thymus nucleic acids, but a variety of fragments of the nucleic acid molecules, which differed widely from each other in structural complexity. Their investigations led to the following conclusions: *a*, Gastric and pancreatic juices are not concerned in nucleic acid disintegrations. *b*, Intestinal juice contains an enzyme (nucleinase) which hydrolyzes tetra-nucleotides into mono-nucleotides, and another enzyme or group of enzymes (nucleotidases) which hydrolyze purine nucleotides into phosphoric acid and nucleosides. The pyrimidine nucleotides are probably not affected by the *succus entericus*. *c*, Extracts of the intestinal mucosa contain nucleinase, nucleotidases for both the pyrimidine and purine nucleotides, and a third enzyme or group of enzymes (nucleosidases) capable of hydrolyzing the purine nucleosides into sugar and

bases. The pyrimidine nucleosides are apparently not hydrolyzed in the intestinal wall. Indeed, studies of Levene and La Forge (193) indicate that they are not hydrolyzed into sugar and bases by extracts of any tissue.

At about the time the work of Levene and Medigreccanu appeared, London and Schittenhelm (208), and London, Schittenhelm and Wiener (209), (210) published the results of feeding experiments in fistula dogs. By means of experiments in which nucleic acid was administered orally, and removed at various stages in its passage through the alimentary canal, the authors arrived at the following conclusions: *a*, The stomach does not digest or absorb nucleic acids. *b*, In the intestine, chemical changes occur in such a fashion as to produce small amount of free purines, but larger amounts of purine compounds of a diffusible nature (nucleotides and nucleosides?). *c*, The exclusion of pancreatic juice from the intestine, or even the extirpation of the stomach or the pancreas, is without effect upon nucleic acid digestion. *d*, The results of *in vitro* experiments agree with the *in vivo* data in showing that pancreatic juice exerts no action, while intestinal juice manifests marked digestive effect upon nucleic acid. It is thus seen that in their essential features, the conclusions of London and his associates are in accord with the findings of Levene and Medigreccanu. It is unfortunate that London, Schittenhelm and Wiener (209), (210) claim to have identified guanosine, and also to have detected a substance having the properties of guanylic acid, following the feeding of thymus nucleic acid. Since guanosine and guanylic acid are products of the disintegration of *yeast nucleic acid*, it is obviously impossible for them to have had their origin in *thymus nucleic acid*. Levene and Jacobs (191) have suggested as a possible explanation of these remarkable observations, that the thymus nucleic acid employed by London, Schittenhelm and Wiener may have been an impure product containing guanylic acid. Nevertheless, the fact that London and his colleagues report such unique findings without attempting to offer a satisfactory explanation for them must necessarily shake one's confidence somewhat in the validity of their other results.

Recently Thannhauser and Dorfmueller (295), (298) have reported that human duodenal fluid splits yeast nucleic acid into uracile nucleotide and a triphosphonucleic acid. It is very probable that their so-called triphosphonucleic acid is a mixture of mono-nucleotides (cf. Jones (130), p. 39). They find (299) that intestinal bacteria disintegrate the purine ring with the liberation of ammonia, and that this accounts for the failure to recover quantitatively purines (or nucleic acids) ad-

ministered *per os*. They believe that under ordinary conditions purines are absorbed probably as nucleosides, and in accord with this conception, they have been unable to detect free purines, other than uric acid, in the blood (297).

There seems to be very little doubt, therefore, that in the alimentary tract nucleic acids are progressively broken down through the intermediary stages of nucleotides and nucleosides. How far these reactions proceed is uncertain. At the completion of the action of the enzymes of the *succus entericus*, and of the intracellular enzymes in the intestinal wall, the products available for absorption are probably phosphoric acid and nucleosides, with more or less sugar (hexose or d-ribose), adenine, and guanine. Such information as we have indicates that all of these products enter the portal circulation. The investigations of Biberfeld and Schmid (39) show that purines are not absorbed via the lymphatics. In animals with thoracic fistulas, these authors were unable to detect the slightest increase in the free or combined purines of the lymph following the feeding of thymus nucleic acid. Indeed, detectable amounts of purines were never found even after hydrolysis of the lymph with 3 per cent sulphuric acid. We are, therefore, forced to assume for the present, that the ingredients of nucleic acids are absorbed by way of the portal circulation. The fate of the purines, with which this article is particularly concerned, will be considered below.

4. THE ANABOLISM OF PURINES. Comparatively little is known concerning the anabolism of purines. The animal organism is certainly not dependent upon preformed purines in the diet for the formation of nucleic acids in the cell nuclei. The observations of Miescher (228) in 1874, that the migrating salmon forms relatively enormous amounts of nuclein for the generation of spermatozoa, indirectly indicates the ability of this animal to synthesize purines. Numerous investigations have confirmed the findings of Miescher, and have established the fact that the salmon, during its long migration from the sea to the spawning grounds, abstains from food, and forms the nuclear material at the expense of its own muscle tissue.

Since the work of Miescher, the problem of purine synthesis has been attacked from several different points of view, and direct and convincing proof has been provided that the cells of both adult and young animals are capable of synthesizing purines to the fullest extent for anabolic purposes. The first to study the question was Tichomiroff (305), who, working with the ova of the silkworm, *Bombyx mori* L., found almost a tenfold increase in purines during the embryonic development of the

eggs. Obviously, these purines must have had their origin in some non-purine ingredient of the ova. Soon after the work of Tichomiroff, Kossel (159) made similar experiments upon hens' eggs, and obtained like results. In spite of numerous attempts, Kossel was unable to detect purines in fresh eggs, but after incubating an egg for fifteen days isolated 8.4 mgm. of guanine and 19.5 mgm. of hypoxanthine (perhaps also adenine). Calculated on a basis of the dry substance in the embryo, the figures amounted to approximately 0.94 per cent of purines. Mendel and Leavenworth (221), using more refined methods, repeated and extended the experiments of Kossel, and obtained uniformly progressive increases in the purine content of both hens' and ducks' eggs during incubation. They succeeded also in isolating and identifying adenine, guanine and hypoxanthine, which are characteristic components of nucleoproteins. These experiments afforded conclusive proof of a synthesis of purines during embryonic development.

Corroboratory evidence of synthesis was supplied by Plimmer and Scott (251) in studies upon the distribution of the various forms of phosphorus in the egg during incubation. They report that the phosphoric acid of the nucleic acid probably has its origin in the vitellin of the yolk. In rats, McCollum (216) has shown that inorganic phosphates may serve as the sole supply of phosphorus for nuclein synthesis (cf. also Fingerling (69)).

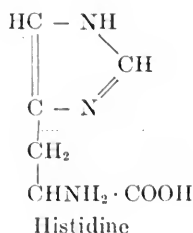
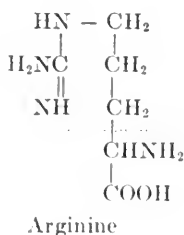
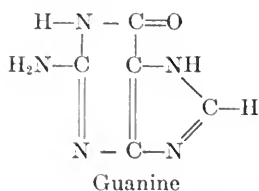
At a somewhat later date Burian and Schur (49) proved the synthesis of purines in growing mammals. Suckling rabbits and pups were permitted to grow for varying periods, and compared as regards their purine content with animals of the same litter which had been killed and analyzed at the beginning of the experiment. In two of the typical rabbit experiments, the increases in purine nitrogen amounted to 94.1 and 136.7 mgm. respectively during eighteen-day periods. The authors were unable to secure sufficient rabbit's milk for the determination of its purine content. Analyses of cow's milk showed the presence of 4.12 to 6.40 mgm. of purine nitrogen per liter. If rabbit's milk contains a similar quantity of purine nitrogen, it is obviously impossible for the increased purine content of the tissues to have had an exclusively exogenous origin. For such to have been the case would have necessitated the consumption of a liter or more of milk per day by each of the growing animals. Nor are the conclusions of Burian and Schur invalidated by the recent determinations of purines in cow's milk by Voegtlin and Sherwin, and Denis and Minot. These authors report that cow's milk contains 5 mgm. of adenine and 10 mgm. of guanine per

liter "as minimum values" (313), and about 15 mgm. of uric acid per liter (59), or a nitrogen content in the form of these three purines of 12.2 mgm. per liter. Even if we assume that the actual purine content of milk is 100 per cent greater than the combined values obtained by Voegtlin and Sherwin, and Denis and Minot, or 25 mgm. of purine nitrogen per liter, we would still be compelled to assume a milk consumption of 200 to 300 cc. per animal per day in order to account for the data of Burian and Schur.

But fortunately we are not limited to evidence of this sort, in which there is the possibility of a marked difference in the purine content of cow's and rabbit's milk (cf. Denis and Talbot (60) for uric acid content of human milk) which might account for the experimental findings. Abundant evidence of an entirely different kind has been accumulated indicating that purines are not necessary articles of diet. As early as 1891 Socin (280), in studying the utilization of inorganic and organic iron compounds, succeeded in maintaining the health and growth of mice upon a purine-free diet of cooked egg-yolk, starch, cellulose, and water. Experiments with some of his animals were continued for sixty to ninety-nine days. Similar conclusions have been reached by McCollum (216) in investigations upon rats. In the elaborate growth experiments of Osborne and Mendel (249), indisputable evidence has been presented that purines are unessential articles of diet, and both they and other investigators (cf. Abderhalden (1)) have verified this fact over and over again in feeding experiments with purified foodstuffs. Moreover, Benedict (32) has made the remarkable observation that a Dalmatian dog, which normally eliminates uric acid in the urine, may be maintained upon a purine-free diet, and continue to eliminate uric acid. In one such experiment a Dalmatian was kept upon a diet devoid of purines for nearly a year, and during this time excreted more than 100 grams of uric acid. Not 10 per cent of this could have come from the preformed purines of the animal's tissues. Thus the power of purine synthesis is not limited to growing animals, but is characteristic of the adult mammal as well.

Finally, Kollmann (151) has recently reported experimental results indicative of purine synthesis in adult man. A healthy young woman was kept upon a constant diet of very low purine content for a period of 50 days. Despite the fact that she gained 4 kgm. in body weight during this time, the uric acid output exceeded the total purine intake by 15 grams for the experimental period. Evidently synthesis of the purine ring must have occurred.

As to the mechanism of purine formation, and the intermediary steps involved, the literature affords very little information. The field is almost entirely unexplored. Obviously, the synthesized purines must have their origin in one or more of the amino-acids of the protein molecule, but the only publication, with which we are familiar, that throws any light upon the problem is the interesting paper of Ackroyd and Hopkins (10). It occurred to these authors that purines might have their origin in arginine and histidine, the pyrimidine portion of the molecule coming from the former, and the imidazole ring from the latter. In this case both arginine and histidine would be necessary for purine synthesis, as indicated in the formulas below.



To test this hypothesis they compared the growth and allantoin output of rats upon diets in which the nitrogenous requirements were supplied respectively by completely hydrolyzed casein, and hydrolyzed casein from which arginine and histidine had been removed. Both types of diet were supplemented with cystine, tryptophane and vitamins in sufficient quantities to render them adequate in these respects for the nutritive requirements of the animals. Upon the ingestion of the diet devoid of the two amino-acids, the animals promptly and rapidly lost weight, and showed decreases of 40 to 50 per cent in the elimination of allantoin. Contrary to the authors' original expectations, however, the addition to the deficient diet of either of the missing amino-acids was followed by a resumption in growth, and an increase in the excretion of allantoin. Furthermore, the decrease in allantoin excretion was much less when either arginine or histidine alone

TABLE 1\*

*Effect of arginine and histidine upon allantoin excretion*

WEEK	MEAN WEIGHT DURING WEEK		ALLANTOINE FROM TWO RATS PER WEEK	NITROGEN PER RAT PER DAY			ALLANTOINE N AS PER CENT TOTAL N	DIET
	Rat A	Rat B		Total N	Purine N	Allantoin N		

Experiment I								
1914	gms.	gms.	mgm.	mgm.	mgm.	m.m.		
1	124	132	458	147	0.65	11.6	7.8	Bread and milk
2	116	126	296	95	0.44	8.0	8.4	Amino-acids without arginine and histidine
3	109	116	260	107	0.30	6.5	6.3	Amino-acids without arginine and histidine
4	105	109	319	116	0.40	8.0	6.7	Amino-acids without arginine and histidine
5	102	106	228	104	0.23	5.8	5.6	Amino-acids without arginine and histidine
6	113	114	347	134	0.36	9.0	6.7	Amino-acids plus arginine and histidine
7	121	124	347	133	0.34	9.0	6.7	Amino-acids plus arginine and histidine
8	131	131	400	157	0.49	10.1	7.0	Amino-acids plus arginine and histidine
9	137	141	454	199	0.69	11.5	5.8	Bread and milk

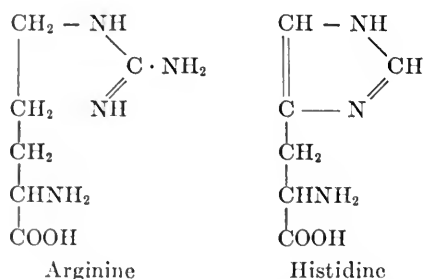
  

Experiment II								
	Rat C	Rat D						
1	146	125	575	182	0.63	14.6	8.0	Bread and milk
2	139	119	406	105	0.44	10.3	9.8	Amino-acids without arginine and histidine
3	125	112	306	111	0.46	7.7	7.0	Amino-acids without arginine and histidine
4	119	106	390	142	0.37	9.9	7.0	Amino-acids without arginine and histidine
5	120	106	310	130	0.39	7.9	6.1	Amino-acids plus arginine and histidine
6	125	115	413	119	0.46	10.5	8.8	Amino-acids plus arginine and histidine
7	131	120	400	135	0.53	9.9	7.3	Amino-acids plus arginine and histidine
8	133	127	486	136	0.50	12.3	9.0	Amino-acids plus arginine and histidine
9	142	137	501	167	0.51	13.6	8.1	Bread and milk

\* Table taken from Ackroyd and Hopkins (10).

was removed from the ration, than when both were excluded. The results of two typical experiments of Ackroyd and Hopkins are reproduced in table 1. The figures for the daily elimination of total-, purine-, and allantoin-nitrogen represent the averages for two rats.

The experimental findings led Ackroyd and Hopkins to modify their original conception, and to conclude that arginine and histidine are replaceable the one by the other in nutrition,<sup>2</sup> and that each may serve as the raw material for the synthesis of the purines. The structural similarity of these two amino-acids is evident from the formulas below.



We shall consider the paper of Ackroyd and Hopkins in another connection later.

There is one other question in connection with purine anabolism which should be briefly considered, namely, the possibility of the utilization of *exogenous* purines for nuclein formation. We have already seen that purines are not necessary ingredients of the diet, but this fact does not exclude the possibility of their being anabolized into nucleic acids *when they are present*, in lieu of a synthesis of purines from the usual precursors. No conclusive answer to this question is to be found in the literature. In a recent paper the writer (262) tentatively suggested that in conditions of physiological stress, such as starvation, the purines liberated in tissue wear and tear might possibly be re-utilized for anabolic purposes. A similar possibility is presented in the case of exogenous purines. While it is exceedingly difficult to exclude such an

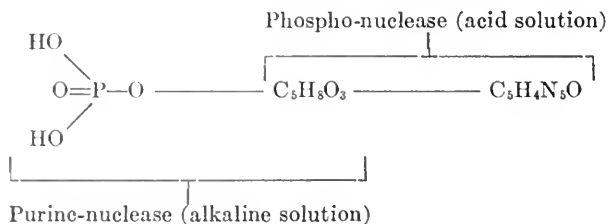
<sup>2</sup> Investigations in this laboratory have served to cast doubt upon the validity of Ackroyd and Hopkins' conclusions regarding the relation of arginine and histidine to growth. Our preliminary experiments indicate that these amino-acids cannot replace each other in nutrition (Rose and Cox (263)). However, our observations do not necessarily affect the deductions of Ackroyd and Hopkins concerning purine formation from the diamino-acids. We expect to present our data in full in the near future.

occurrence in the case of at least a part of the ready-formed purines of the diet, it must be admitted that the evidence available is probably unfavorable to such a view. The prompt increase in the elimination of uric acid or allantoin following the ingestion of nucleic acids or purines (cf. Krüger and Schmid (171), Schittenhelm and Bendix (270), Mendel and Brown (220), Mendel and Lyman (222)), would seem to indicate that the latter are promptly catabolized, and their end-products discarded. On the other hand, it is a matter of common experience, as will be discussed later, that purines administered either orally or parenterally are not quantitatively recovered. The fate of the uneliminated portion is unknown.

5. THE CATABOLISM OF PURINES. *a. The chemical transformations and the enzymes involved.* Nucleic acids liberated in the disintegration of nucleoproteins of animal tissues are believed to be hydrolyzed into their ultimate components in a manner quite similar to that which occurs in the case of exogenous nucleic acids during digestion in the alimentary tract. However, more profound decomposition is brought about under the influence of the intracellular enzymes than is occasioned by alimentary ferments, and the process is more complex inasmuch as a larger number of enzymes is involved, and a greater variety of transformations is thus made possible. In the catabolism of nucleic acids and the component purines, one sees a remarkable illustration of specificity in enzymatic reactions. Nowhere else in metabolism is the division of physiological labor between several catalyzers so beautifully exemplified as in the disintegration of nucleic acids.

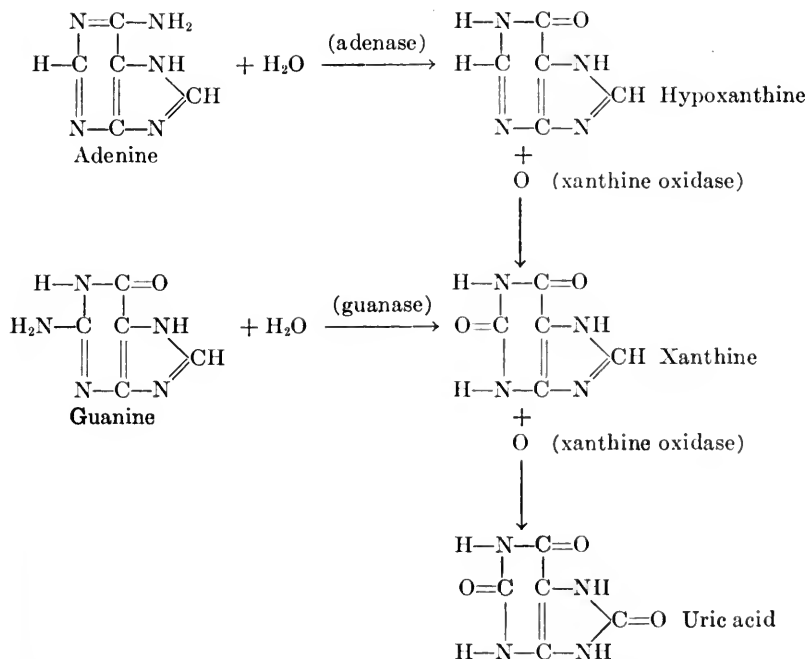
According to Levene and Medigreceanu (196), (198), practically all organs of the body, as far as they have been examined, contain nucleinases which hydrolyze poly-nucleotides into mono-nucleotides, nucleotidases which decompose mono-nucleotides into phosphoric acid and nucleosides, and nucleosidases which split nucleosides into their component sugar and base. Inasmuch as the pyrimidine nucleotides and nucleosides are hydrolyzed by ferments which are different from those which decompose the purine nucleotides and nucleosides, it seems quite probable that four specific ferments of each class are concerned in nucleic acid disintegration. But other paths of decomposition may also occur besides those proven by Levene and Medigreceanu. Leaving the pyrimidine nucleotides out of consideration, Jones (128), (129) has shown that the purine nucleotides may undergo enzymatic disintegration in two different ways. Phosphoric acid may be removed leaving the nucleosides, or the purine bases may be removed leaving

the sugar and phosphoric acid combined. Amberg and Jones (14) have named the first of these enzymes "phospho-nuclease" (nucleotidase of Levene and Medigreceanu), while they designate the second as "purine-nuclease." Phospho-nuclease acts most rapidly in a faintly acid solution, and purine-nuclease is most effective in a faintly alkaline solution (130).



Furthermore, Jones (129) and Amberg and Jones (14) have shown that deamination of the purines may occur before they have been liberated from the nucleosides. Thus adenosine may be transformed into inosine, and guanosine into xanthosine. The enzymes responsible for these changes are known as adenosine-deaminase (14), and guanosine-deaminase (129) respectively. Other ferments, which Jones and his associates call adenosine-hydrolase (15), guanosine-hydrolase (134), xanthosine-hydrolase (129), and inosine-hydrolase (14) respectively, hydrolyze the corresponding nucleosides into their component purines and sugar. Thus the path of nucleic acid decomposition may vary with conditions, and with the enzymes available in the organ or tissue in question.

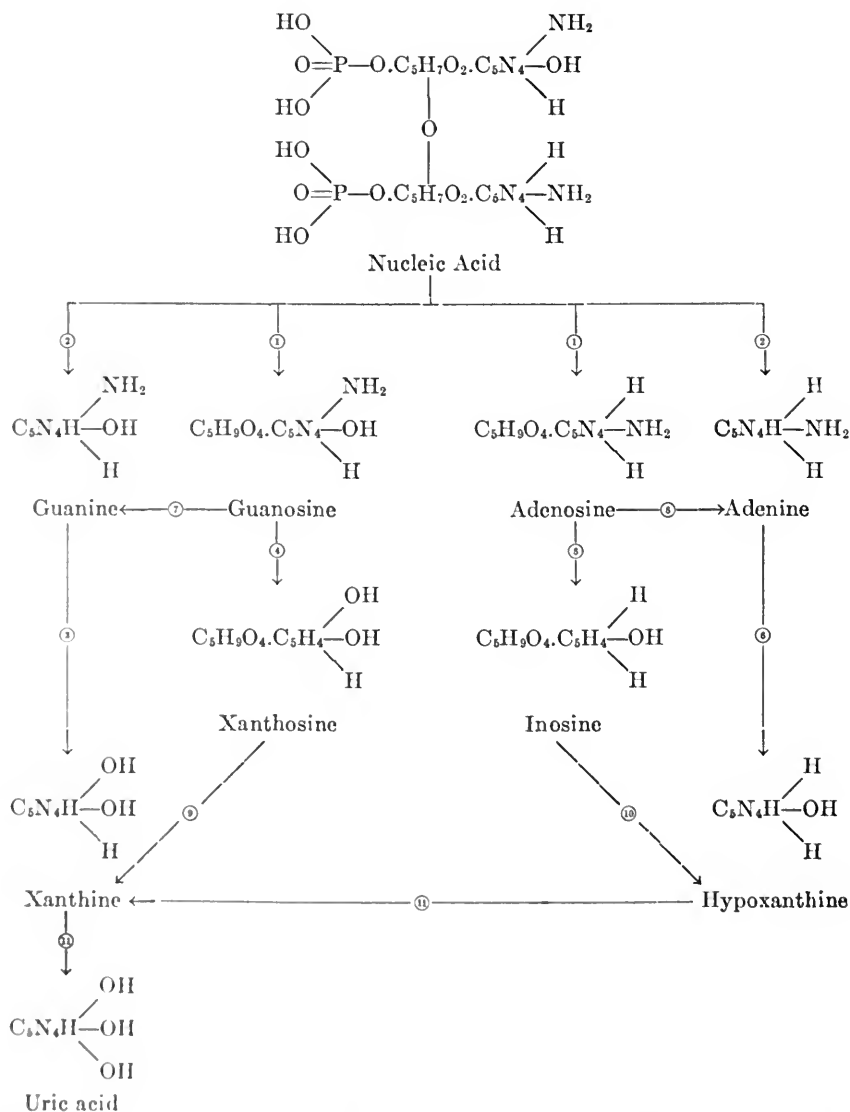
The purines liberated in these reactions, as well as those produced by the decomposition of the mono-nucleotides (inosinic and guanylic acids) of muscular, pancreatic, and other tissues, are believed to be oxidized to uric acid through the instrumentality of the enzymes adenase (140), guanase (136), and xanthine oxidase (282), (329), as indicated in the formulas below.



All of the transformations briefly described above have been diagrammatically summarized by Amberg and Jones (14) in a scheme which is reproduced on page 564. Each encircled number refers to the enzyme responsible for the change in question. The nucleic acid is represented as the di-nucleotide of adenylic and guanylic acids united by ether linkage through the sugar molecules. There appears to be no doubt of the independent existence of each of the ferments listed.

The distribution of purine enzymes varies widely in different species, and in different organs of a given species, and cannot be discussed in the space available for this article. It is noteworthy, however, that **adenase** is not supposed to be present in the human body (Winternitz and Jones (334)), with the possible exception of the mixed organs of embryos (Long (211)). It is probable, therefore, that under ordinary conditions in man adenine is deaminized before it is liberated from its nucleoside.<sup>3</sup> For further information concerning the occurrence of purine ferments, reference should be made to the monograph of Jones (130). A resumé of the literature will also be found in the publications of Bloch (41), (42), (43).

<sup>3</sup> An interesting paper by H. Jackson has just appeared (Journ. Biol. Chem., 1923, lvii, 121) in which the author presents evidence indicating the occurrence of *adenine nucleotide* in human blood. He believes that 15-25 mgm. per 100 cc. are normally present, and that this compound accounts for a considerable part of the undetermined nitrogen of the circulating fluid.



(1) Phospho-nuclease (Amberg and Jones (14)), or nucleotidase (Levene and Medigreceanu (198)).

(2) Purine-nuclease (Amberg and Jones (14)).

(3) Guanase (Jones and Partridge (136)).

(4) Guanosine-deaminase (Jones (129)).

(5) Adenosine-deaminase (Amberg and Jones (14)).

(6) Adenase (Jones and Winternitz (140)).

(7) Guanogine hydrolase (Jones and Belt (134)), or nucleosidase (Levene and Medigreceanu (198)).

(8) Adenosine hydrolase (Amberg and Jones (15)), or nucleosidase (Levene and Medigreceanu (198)).

(9) Xanthosine-hydrolase (Jones (129)).

(10) Inosine-hydrolase (Amberg and Jones (14)), or nucleosidase (Levene and Medigreceanu (198)).

(11) Xanthine oxidase (Spitzer (282), Wiener (329)).

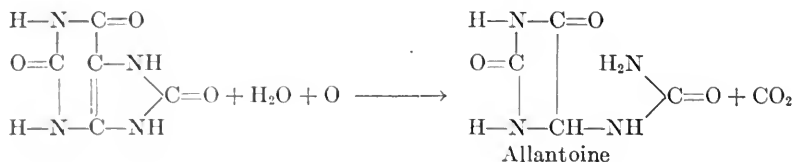
In connection with the enzymes concerned in purine metabolism, should be mentioned the interesting discovery of recent data by Jones (132), (133), of a thermostable catalyst (enzyme?) in extracts of pig's pancreas. The pancreas of the pig contains a variety of active agents which may be instrumental in the hydrolysis of nucleic acid. According to Jones, all of these are destroyed by boiling except one, viz., the one which acts upon the nucleotide bindings of yeast nucleic acid. When brought in contact with yeast nucleic acid, the ferment acts quite rapidly at first, more slowly afterwards, but causes the decomposition of the tetra-nucleotide without the liberation of either phosphoric acid or purines. It is much more active at 40° than at 20°, and exhibits about the same activity in amphoteric, faintly alkaline, or faintly acid reaction. It has not been detected in extracts of any other organ or tissue, nor does it decompose thymus nucleic acid. As stated by Jones (133), the fact that the spleen, liver and other tissues of the pig do not contain the thermostable agent, and yet are able to disintegrate poly-nucleotides, indicates that either there are two ferments, one of which is thermolabile, or that the decomposition of nucleic acid by tissue extracts does not proceed along conventional lines.

At first Jones believed that the heat-stable enzyme completely disintegrates the nucleic acid into its four component nucleotides. More recently Jones and Perkins (137) have found that this is not the case. They state that the active agent acts upon nucleotide linkages only, but that it does not split the nucleic acid into mono-nucleotides, since "the end product contains intermediate substances between nucleic acid and mono-nucleotides." The rupture produces no increase in titratable acidity, in contrast to the effect of 1 per cent sodium hydroxide, which at room temperature also decomposes nucleic acid, but with appreciable increase in titratable acidity (Jones and Perkins (138)). This difference in behavior of nucleic acid under the influence of the two agents, thermostable enzyme and 1 per cent alkali, indicates, according to Jones, that at least one of the nucleotide linkages of yeast nucleic acid is through the carbohydrate molecules, and has led him to suggest his latest formula for nucleic acid, which is given above (p. 550).

It is impossible at the present time to offer any explanation for the presence of the thermostable agent. Future investigations of Jones along this line will be followed with great interest. Catalyzers resistant to heat have not often been reported in the animal body. Hammett (102) has recently found in muscle tissue a thermostable agent capable of transforming creatine into creatinine, an observation which we have

been able to verify in this laboratory (264). Perhaps future investigations will indicate that such catalysts are not as rare as our present lack of knowledge concerning them would seem to indicate.

*b. The physiological oxidation of uric acid (uricolysis).* In most mammals the uric acid arising in purine catabolism by reactions outlined above is not eliminated as such, but undergoes further oxidation, probably with the production of allantoin.



Evidence of the occurrence of this reaction was presented by Salkowski (266), who observed the elimination of allantoin in dogs following the administration of uric acid. Corroboration of his findings was provided by a number of investigators, notably Cohn (53), Minkowski (232), Mendel and Brown (219), Mendel and White (225), and Wicichowski (325). Underhill and Kleiner (309) first showed that the urine of fasting dogs contains allantoin, thus proving that it is not derived exclusively from dietary purines. Furthermore, extracts of tissues have been shown to possess the power of uric acid destruction (cf. Brunton (47), Wiener (329), (330), (332), and Wicichowski (324)), due to the presence of an enzyme, uricase, located chiefly in the liver, and occasionally in the spleen and kidneys of lower animals (Wells (317), Battelli and Stern (25)). By means of perfusion experiments, Ackroyd (6), (8) has shown that the surviving liver of the rabbit quantitatively transforms uric acid into allantoin. Allantoin has been detected also in the blood of lower animals (Hunter (115)); and is the end-product, rather than an intermediate stage, of purine metabolism in these forms, as evidenced by the fact that it undergoes no further oxidation when administered preformed (Ackroyd (6), Taylor and Adolph (292)).

In man uric acid is believed to be the end-product of purine metabolism. Minkowski (232) was unable to detect allantoin in human urine, even after purine administration. More recent investigations (Wicichowski (326), (327)) have shown that small amounts of this substance (10 to 25 mgm. per day) are present in the urine of man, but doubtless have their origin in preformed allantoin of the food (Ackroyd (7)). As might be anticipated from these facts, attempts to demonstrate the presence of uricase in human tissues have been unsuccessful (Battelli

and Stern (25), Miller and Jones (229), Wells and Corper (321), (322), Wiechowski (326)). From the evolutionary point of view, it is of interest to note that anthropoid apes also neither excrete allantoin nor contain the uricolytic enzyme in their tissues (328), (320). Monkeys, on the other hand, are provided with uricase (317), and eliminate allantoin as the chief product of their purine metabolism (116), (117). In this respect monkeys appear to be more closely related generically to the lower animals than to man or the ape. The unique position occupied by man and the ape, as regards purine metabolism, is beautifully

TABLE 2\*  
*The "uricolytic indexes" of mammals*

ORDER AND SPECIES	INDEX	ORDER AND SPECIES	INDEX
Marsupialia:		Ungulata:	
Opossum.....	79	Cow.....	93
Rodentia:		Horse.....	88
Rat.....	96	Sheep.....	80
Mouse.....	98	Goat.....	92
Guinea pig.....	94	Pig.....	98
Rabbit.....	95	Proboscidea:	
Carnivora:		Elephant.....	72
Raccoon.....	95	Primates:	
Black bear.....	94	Monkey.....	89
Badger.....	98	Chimpanzee.....	0
Cat.....	97	Man.....	2
Coyote.....	97		
Dog.....	98		
Dingo dog.....	96		
Dalmatian coach dog.....	32		

\* Table taken from Hunter and Ward (120).

illustrated in table 2, taken from Hunter and Ward (120). Hunter and his associates (119), (118), (120) have determined the so-called "uricolytic index" for twenty-two species of animals. This index is the ratio of the output of allantoin nitrogen to the sum of the output of allantoin and uric acid nitrogen. The figures indicate that while the uricolytic indexes for man and the ape are practically zero, these values for other mammals range from 32 to 98.

Although uricolysis in the ordinary meaning of the term has not been possible of demonstration in man, and as pointed out by Fine (64) would seem to be contraindicated by the presence of relatively large

amounts of uric acid in human blood and tissues, the common experience of workers in this field is that administered purines are not quantitatively recovered in the excreta, either as uric acid or as unchanged purines (cf. Kikuchi (144)). This fact is exemplified in a carefully controlled experiment of Taylor and Rose (293). The subject of the experiment, a healthy young adult, was placed upon a diet of exactly 10 grams of nitrogen and 2,000 calories per day. During the first three days, milk, eggs, starch and sugar were the sole foods. For the following three days, a fraction of the milk-egg was substituted by sweetbreads containing the same amount of nitrogen. During the next four days, this substitution was doubled; and during the last four days the subject

TABLE 3\*

*Thymus feeding and uric acid excretion in man*

	PERIOD I 3 DAYS	PERIOD II 3 DAYS	PERIOD III 4 DAYS	PERIOD IV 4 DAYS
Total urinary N.....	8.90	8.70	9.10	8.80
Urea N + Ammonia N.....	7.30	7.10	7.10	7.05
Creatinine N.....	0.58	0.55	0.56	0.47
Total purine N.....	0.11	0.17	0.26	0.10
Uric acid N.....	0.09	0.14	0.24	0.07
Rest N.....	0.91	0.88	1.18	1.18
Nitrogen input.....	10.0	10.0	10.0	10.0
Purine N input.....	0	0.17	0.34	0
Average fecal N output.....	0.5	0.5	0.5	0.5

\* Table taken from Taylor and Rose (293).

was again placed upon the diet of the first period. Thus the nitrogen input of the tests was derived as follows:

First period: milk-egg nitrogen, 10 grams.

Second period: milk-egg nitrogen, 7 grams; sweetbread nitrogen, 3 grams.

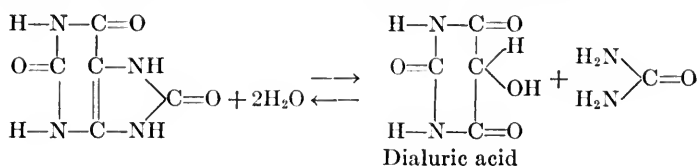
Third period: milk-egg nitrogen, 4 grams; sweetbread nitrogen, 6 grams.

Fourth period: milk-egg nitrogen, 10 grams.

Table 3 presents the results of the experiment in terms of nitrogen daily. Judging by the regularity in output of fecal nitrogen, the digestion and absorption of the sweetbreads was normal and complete. Tests of the feces for purines never showed the presence of more than minute traces. The possibility of bacterial decomposition of purines

with the production of ammonia, as suggested by Thannhauser and Dorfmueller (299), is not excluded, but if such a disintegration occurred to an appreciable extent in our experiment, it is surprising that the urinary urea-ammonia output was not increased. It is clear that the increments in urinary purine nitrogen represent less than half of the known input. The data raise the important question as to whether uric acid may not be oxidized in the human organism without the production of allantoin. Certainly the possibility of such an occurrence has not been excluded.

In regard to uricolysis, the investigations of Ascoli and his associates (18), (19), (38), (123), (125), (254) require brief consideration. These investigators report that incubated liver tissue of animals destroys uric acid with the production of dialuric acid and urea; and in an atmosphere of carbon dioxide reforms uric acid from the disintegration products. The reversible reaction involved is represented as follows:



They believe that the active agents responsible for uric acid formation are a thermostable body in the tissues, and a thermolabile enzyme in the blood, both of which are said to be necessary for the synthesis (124). These observations are difficult to correlate with the known facts of purine metabolism. If true, they serve to complicate the situation as regards allantoin, for according to the Italian investigators, this substance, in contrast to dialuric acid, is neither formed from nor transformed into uric acid under the conditions of their experiments. Recently Spiers (281) has denied Ascoli's results *in toto*. It must be admitted, however, that in studies of this sort positive findings are of much greater value than are negative results. The entire question should be reinvestigated.

An interesting racial anomaly of purine metabolism was discovered by Benedict (32) in the case of the Dalmatian dog. In contrast to other dogs, the Dalmatian, upon a purine-free diet, excretes almost as much uric acid per day as does a man upon a similar régime. Benedict has shown that uric acid injected subcutaneously is quantitatively excreted as such in the urine. Wells (319) and Givens (quoted by

Wells) have confirmed Benedict's findings, and the former has made a study of the purine enzymes in the Dalmatian hound. He finds that uricase is present in the liver, indicating that the excretion of uric acid is not due to the absence of the uricolytic ferment. Watanabe (314) also has reported analyses upon two such dogs. He found uric acid in the urine, but in much smaller quantities than observed by other investigators. Possibly his dogs were not of pure Dalmatian breed. Onslow (248) has recently shown that hybrids resulting from the cross of a Dalmatian with another breed of dog excrete uric acid and allantoin in the same proportions as ordinary dogs. According to this author, the greater power of destruction of uric acid is a dominant hereditary character. Benedict's discovery of the metabolic peculiarities of the Dalmatian is not only of scientific interest, but is also of extraordinary practical importance in that it provides an experimental animal whose purine metabolism is similar to that of man. Reference will be made in another connection to Benedict's studies upon the Dalmatian.

*c. The fate of exogenous purines.* The literature concerning the fate of ingested purines and nucleic acid contains a great many contradictions. The differences of opinion expressed by early investigators are doubtless to be accounted for by the inadequate methods of analysis employed in the separation and identification of the purines, by the use of different species of animals with variable enzyme equipment, and by the failure to appreciate the relationship of allantoin to purine metabolism. Thus Stadthagen (283) and Gumlich (98) observed no increase in the output of uric acid in dogs following the feeding of yeast nucleic acid. The former sought for allantoin in the urine, but failed to detect the slightest trace, while apparently the latter did not consider allantoin formation as a possibility, despite the fact that this substance had been known as an ingredient of urine since the days of Wöhler (335). Later investigations, as will be seen below, have shown that in most mammals uric acid excretion is somewhat increased, and allantoin excretion greatly increased by nucleic acid feeding, but it is not surprising that the small increments in the former should have been overlooked in many experiments on account of the inaccuracies of the analytic methods. Nevertheless, increases in uric acid elimination following the feeding of nucleic acids, or foods containing them, were observed in man by Richter (258), Kühnau (172), Weintraud (315), Umber (306), Hess and Schmoll (106), Weiss (316), Jerome (126), Burian and Schur (51), Loewi (207), Mendel, Underhill

and White (224), and others. Recently Thannhauser and Schaber (302) have reported that following the injection of 1 mgm. doses of adenosine and guanosine in man, recoveries of the purines in the form of uric acid varied in four of the five subjects between 88.8 and 119.9 per cent. The fifth subject, an individual from a gouty family, though he himself had no clinical symptoms of the disease, excreted in two experiments extra uric acid to the extent of only 47.3 and 61.3 per cent of the administered purine. Comparable recoveries of injected nucleosides had previously been obtained in man by Thannhauser and Bommes (296). Less pronounced increases in uric acid following nucleic acid feeding were observed in animals by several investigators, notably Cohn (53) and Mendel and Brown (219), who were also able to demonstrate marked increases in the output of allantoin. Burian and Schur (51) first emphasized the fact that urinary uric acid is derived partly from body cells and partly from the diet, and named these two fractions "endogenous" and "exogenous" uric acid respectively.

In regard to the fate of free purines in the diet, pioneer investigations yielded results even more chaotic than in the case of nucleic acids. Administration of guanine or xanthine to rabbits and dogs was said to be without effect upon uric acid excretion by Kerner (143), Nencki and Sieber (241), Baginski (22), and Krüger and Salomon (167). Even Burian and Schur (51), despite their important observations concerning the effect of exogenous nucleic acids, failed to detect an increase in uric acid output following guanine feeding in man. Hall (100) was inclined to believe that the negative results were due to the relative insolubility of the purines employed, which limited their absorption. That this point was well taken is indicated by the subsequent observations of Schittenhelm and Bendix (270). These authors obtained increases in the output of uric acid following the parenteral administration of guanine in rabbits. Nevertheless, the idea became more or less current that free purines, if catabolized at all, are not transformed to the same end-products as are combined purines (nucleic acids). On the contrary, Minkowski (231) and v. Mach (212) proved that the subcutaneous administration of hypoxanthine in geese causes a marked increase in urinary uric acid. Their experiments are of particular interest in that they constitute the first demonstration of the ability of the animal organism to transform a free oxy-purine into uric acid. In later investigations Minkowski (232) showed that in dogs and men, as well as in birds, hypoxanthine is a precursor of uric acid. Horbaczewski

(111), (112) obtained similar results with hypoxanthine and xanthine. Unfortunately he erred in believing that the leucocytes are the sole source of urinary uric acid, and that the effect of the hypoxanthine or xanthine administration was an indirect one in that purines were supposed by him to occasion a leucocytosis.

Confirmatory evidence that exogenous purines, whether free or combined, are transformed into uric acid (or allantoin) was provided by Krüger and Schmid (171). Mendel, Underhill and White (224), and Mendel and Lyman (222). Mendel, Underhill and White showed that the adenine and guanine of nucleic acid are deaminized and oxidized to uric acid even when the nucleic acid is administered per rectum. Mendel and Lyman found that the oral administration of purines in man led to increases in the output of uric acid amounting to about 60 per cent of the hypoxanthine, 50 per cent of the xanthine, 25 per cent of the guanine, and 34 per cent of the adenine. The adenine experiments of Mendel and Lyman are of particular interest in view of the supposed absence of adenase in the tissues of man. Inspection of their data affords no opportunity of explaining the increases in uric acid output on the basis of a toxicity leading to an exaggerated endogenous nuclear catabolism. The increases in nitrogen elimination following adenine administration are no greater than may be accounted for by the nitrogen present in the purine itself. Nephritic symptoms, accompanied by the formation of 6-amino-2-8-dioxy-purine and its deposition in the kidneys, have been reported in animals following adenine administration (cf. Minkowski (232), Nicolaier (247), Ebstein and Bendix (61)). No abnormal effects were observed in Mendel and Lyman's experiments upon men, but occasionally albuminuria was detected in some of their animal investigations. It may be possible for the organism, in the absence of adenase, to catabolize adenine to uric acid by oxidation in positions 2 and 8 followed by hydrolytic deamination, instead of by direct deamination of the purine with the production of hypoxanthine.

In an interesting paper concerning the fate of free purines in the organism of the monkey, Hunter and Givens (117) have shown that the subcutaneous administration of adenine, guanine, hypoxanthine, or xanthine, as well as of sodium nucleate, leads in each case to slight increases in the output of uric acid and free purines, and to a marked increase in the elimination of allantoin. Recoveries of the injected purines varied from 69 to 105 per cent. The significant data are reproduced below in table 4. The figures in the last column represent

approximations only. In the case of guanine, adenine, and sodium nucleate, account is taken of the fact that uric acid and allantoin contain but four-fifths of the original amino-purine nitrogen; and of the two alternatives offered in the table for the percentage recovery, the lower figures rest upon the assumption that the extra bases are amino-purines, while the higher assume the presence of oxy-purines. The data clearly show that in the monkey free purines of the diet are catabolized, at least in part, just as are combined purines of nucleic acids.

TABLE 4\*  
*Catabolism of exogenous purines in the monkey*

PERIOD	SUBSTANCE	PURINE N ADMINIS- TERED	RECOVERED				Per cent of theory
			Milligrams nitrogen				
			Allan- toine	Uric acid	Bases	Total	
		<i>m. m.</i>					
80	Uric acid.....	36	8	14		22	61
110	Uric acid.....	49	12	23		35	71
89	Guanine.....	71	38	2	20	60	99-105
100	Xanthine.....	56	35	2	17	54	97
97	Adenine.....	64	15	3	21	39	69-76
85	Hypoxanthine.....	50	28	3	9	40	80
107	Sodium nucleate.....	90	38	3	26	69	88-96

\* Table taken from Hunter and Givens (117).

The investigations of both Mendel and Lyman and Hunter and Givens serve in the main to emphasize the probability that both endogenous and exogenous purines are catabolized through the same intermediary stages, with the production of identical end-products. The source of the purines, whether in cellular catabolism or in absorption from the alimentary tract, would seem to play no part in determining how oxidation shall occur. However, certain experiments reported in the literature are difficult to interpret on the basis of a single catabolic path for endogenous and exogenous purines. Indeed, Hunter and Givens' experiments with uric acid are of interest in this regard. As will be observed in table 4, the injection of uric acid in the monkey led to a comparatively small increase in the output of allantoin, but to a very decided increase in uric acid excretion. In both of the experiments recorded, about 40 per cent of the uric acid reappeared unchanged in the

urine. From the high uricolytic index of the monkey, and from the behavior of the purines other than uric acid, one would have expected a larger proportion of the latter to have been oxidized to allantoin. The authors suggest that the high output of unaltered uric acid may be related to the fact that in the monkey uricase is confined to one organ, namely, the liver (cf. Wells (317)). They believe that parenterally

TABLE 5\*

*Thymus feeding and uric acid injection in the Dalmatian dog*

TOTAL N	UREA + NH <sub>2</sub> N	URIC ACID N	ALLAN- TOINE N	INCREASE IN URIC ACID N	INCREASE IN ALLAN- TOINE N	REMARKS
<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	
4.37	3.86	0.120	0.050	0.223	0.480	Normal diet
4.54	4.03	0.125	0.050			
4.82	4.36	0.127	0.050			
6.48	5.87	0.177	0.210			
6.86	6.15	0.197	0.210			Same diet + 100 gms. thymus daily = 0.32 gms. purine N
7.30	6.58	0.200	0.210			
5.75	5.27	0.129	0.058	0.223	0.480	Normal diet
5.54	5.08	0.132	0.058			
5.27	4.84	0.137	0.058			
5.18	4.50	0.290	0.115			Same diet + 500 mgm. of uric acid subcu- taneously
5.13	4.47	0.299	0.142			
5.00	4.30	0.305	0.133			
5.02	4.54	0.124	0.066			

\* Table taken from Benedict (32).

introduced uric acid may escape contact with the enzyme before elimination has occurred. On the contrary, if this be true it is exceedingly difficult to understand why uric acid formed from injected purine bases does not likewise escape oxidation.

Another observation of interest in this connection is that of Benedict (32) upon the purine metabolism of the Dalmatian coach hound. This author compared the effect of thymus feeding and uric acid injection upon urine composition. As will be seen in table 5 taken from Benedict's article, thymus feeding caused a much larger increase in the output of allantoin than of uric acid. On the purine-free diet the

uric acid nitrogen was more than double that of the allantoin, while after the thymus ingestion the increase in uric acid nitrogen was only about one-half the increase to be found in the allantoin nitrogen. These data, as pointed out by Benedict, may perhaps be interpreted as indicating different paths of catabolism of endogenous and exogenous purines. Furthermore, the subcutaneous administration of uric acid in Benedict's experiment was followed by a marked increase in the allantoin output, despite the fact that the uric acid was quantitatively recovered as such in the urine. Evidently the uric acid administration induced an increased allantoin excretion. On the basis of these results Benedict states that "it seems probable that uric acid and allantoin are interrelated in metabolism in other ways than have been heretofore assumed." Results somewhat analogous to those of Benedict had been obtained previously in a different species of animal by Ackroyd (9). This investigator found a rise in the output of allantoin in the rat following the injection of hypoxanthine, although Rohdé and Jones (260) were unable to detect xanthine oxidase in the total mixed tissues of the rat. Jones (130) remarks that "it is conceivable that hypoxanthine can 'induce' allantoin in the organism of the rat" in a manner analogous to the effect of uric acid upon allantoin elimination in the Dalmatian dog. The situation regarding purine metabolism in the rat is further complicated by the fact that the urine of this animal habitually contains rather large quantities of uric acid (cf. Folin and Morris (86)). To account for the presence of the latter, one must assume its formation by means of reactions which do not involve xanthine oxidase.

Finally must be mentioned the work of Hirokawa (108), who showed that the feeding of thymus tissue to dogs is followed by an excretion of allantoin which is almost quantitative, while the increase in uric acid is very small. If, however, the high purine ration is continued for several weeks, there results a gradual decrease in allantoin, accompanied by a tenfold increase in uric acid.

The work of Benedict, Ackroyd and Hirokawa serves to emphasize the unsatisfactory state of our knowledge of purine metabolism, and to point to the necessity of further investigations before answers will be found to the many questions connection with the catabolic fate of free and combined, endogenous and exogenous purines.

*d. The excretion of uric acid and purines.* The uric acid arising in the catabolism of endogenous and exogenous purines is in man excreted in the urine chiefly as mono-urates. The total output per day depends

upon the quantity of purines or purine-containing foods ingested in the diet. On a purine-free ration it usually amounts to 0.30 to 0.50 grams *per diem* (cf. Folin (77)), but is subject to fairly wide variations in different individuals. Indeed, in the same individual the endogenous uric acid elimination is not as constant from day to day as was once believed. The quantity and kind of purine-free food play an important part in determining the output. This will be discussed in another connection.

Excretion is less during the night than during the day (259), (174), (52). Determinations of the hourly elimination show that the maximum output is reached in the fore-noon, and is followed by a gradual decline until the very early morning, when an increase to the maximum again occurs (244), (52).

Diseases in which there is an exaggerated nuclear catabolism also usually lead to more or less marked increases in the excretion of endogenous uric acid. Two illustrative conditions of this sort are leucemia (142), (272), and pneumonia (142).

At various times in the past muscular activity has been said to increase the output of endogenous uric acid (cf. Burian 48)). This idea, which had been practically abandoned, has been revived in recent years by Raiziss, Dubin and Ringer (256), and Kikuchi (145). Rakestraw (257) reports slight increases in the uric acid of the blood following exercise. Probably the balance of evidence, however, is against the view that moderate exertion influences purine metabolism. Unquestionably, work of a very strenuous or unaccustomed nature may increase uric acid elimination.

In addition to uric acid, human urine contains small quantities of other purines, the total amount approximating 15 to 45 mgm. per day (Flatow and Reitzenstein (76)). We are indebted to Krüger and Salomon (168), (169) for our knowledge of these purines. From 10,000 liters of urine these investigators prepared 94.49 grams of purine bases having the following composition:

1-7-Dimethyl xanthine.....	15.310 grams
1-Methyl-xanthine.....	31.285 grams
7-Methyl-xanthine.....	22.345 grams
7-Methyl-guanine.....	3.400 grams
Adenine.....	3.540 grams
Hypoxanthine.....	8.500 grams
Xanthine.....	10.110 grams
	<hr/>
	94.490 grams

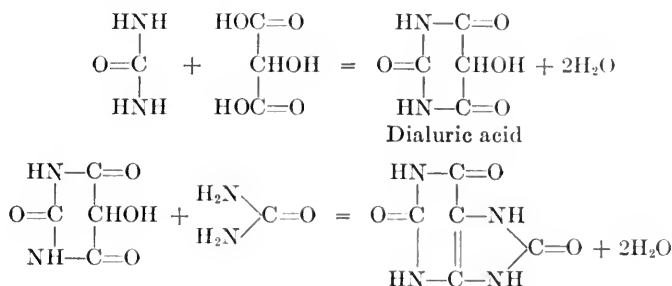
As will be observed, more than two-thirds of the total bases were methyl-xanthines, which do not occur as ingredients of nucleic acids. Evidently they have their origin in the three methyl-purines of beverages, namely, caffeine of coffee, theobromine of cocoa, and theophylline of tea. These substances, which are respectively 1-3-7-trimethyl-xanthine, 3-7-dimethyl-xanthine, and 1-3-dimethyl-xanthine, undergo partial demethylation in the body with the production of mono- and di-methyl-purines (cf. Albanese (11), (12), Bondzynski and Gottlieb (44), (45)). Apparently in man as in the rabbit (166), the methyl group in position 3 is most readily removed, and that in position 7 is most difficult to remove. The methyl group in position 1 is intermediate in stability. Demethylation of caffeine, theobromine, the theophylline in position 3, respectively, doubtless accounts for the methyl-purines of the urine. In the dog the conditions are the reverse of those which pertain in man and the rabbit. In this species (165) demethylation is most readily accomplished in position 7, and next in the case of position 1. The methyl group in position 3 is most resistant. Methyl-purines other than the three which occur naturally, and even dioxy-purines other than xanthine, may undergo no change whatever in passage through the organism (94). This fact emphasizes the observation made above, that purine enzymes are remarkably specific in their action.

Numerous attempts have been made in the past to show an increase in uric acid elimination in man and animals following the administration of methyl-purines. Most of these experiments have yielded negative results (232), (50), (170), (273). Taylor (290) observed increases in uric acid following caffeine ingestion. Recently, Benedict (32) obtained a distinct rise in uric acid excretion, both in man and the Dalmatian dog, following the administration of caffeine. It appears that in the case of this methyl-purine at least, a small part is completely demethylated and oxidized to uric acid, and that the remainder is excreted as the mono- and di-methyl-xanthines. Perhaps the most interesting feature of Benedict's caffeine investigation was the tendency to nitrogen retention observed in both the human and dog experiments. Even comparatively small doses led to distinct falls in nitrogen output. Obviously, there is a twofold reason why methyl-purines should be excluded from the diet of gouty individuals.

As regards the amino- and oxy-purines of human urine, the analysis of Krüger and Salomon shows the absence of guanine, the presence of relatively large amounts of xanthine and hypoxanthine, and of smaller amounts of adenine. Probably the presence or absence of a purine in the urine may be correlated with the occurrence and distribution of



But according to Wiener (331), the avian liver oxidizes lactic acid to tartronic acid, and condenses the latter with urea as indicated in the equations below:



Certainly this method of uric acid formation does not occur extensively in mammals. On the other hand, the possibility of traces of purines arising in this fashion has not been excluded.

6. THE URIC ACID OF THE BLOOD. Most of the modern investigations of purine metabolism have been directed toward determining the changes in the uric acid content of blood in normal and pathological conditions. Until the past decade our knowledge of blood uric acid was limited practically to the data of Garrod. Between 1848 and 1858, Garrod (90) published several interesting communications in which he described the isolation of uric acid from the blood, first accomplished by him, and pointed out that while normal blood contains only traces of uric acid, the blood of gouty or nephritic individuals shows the presence of several milligrams per 100 cc. He proved that contrary to the current belief at that time, no increase in blood uric acid occurs in rheumatism. Following the work of Garrod, almost no progress was made along this line until 1913, when Folin and Denis (80) devised their colorimetric method for uric acid estimation. This method, and the several improved procedures of Folin (82), (87), (88), (79), Benedict (36), (30), (33), (35), (34), and others (239), (234), applicable to both blood and urine, have been the means of greatly increasing our knowledge of purine metabolism. Folin and Denis (81) found the normal uric acid content of human blood to vary between 0.7 and 3.7 mgm., with an average not far from 2 mgm. per 100 cc. These values have been verified over and over again by other investigators. Thus McLester (217) reports values of 0.5 to 2.9 mgm. per 100 cc. of blood in 15 normal persons on purine-free diets, and Gettler and Baker (91) obtained values of 1 to 3.5 mgm. per 100 cc. in 30 normal individuals. The average normal concentration

is undoubtedly close to 2 mgm. per 100 cc. of blood, but values as low as 0.7 and as high as 3.5 occasionally occur in perfectly healthy individuals. The ingestion of purine-containing foods is usually without effect upon blood uric acid in normal individuals, but occasions more or less marked increases in persons with renal insufficiency (57). On the contrary, the ingestion of excessive amounts of yeast is said to cause a rise in blood uric acid even in normal subjects (89). In infants during the first few days of life, while uric acid excretion is unusually large, the concentration in the blood is likewise high, generally varying between 3 and 5 mgm. per 100 cc. (148). It is present in fetal blood in amounts equivalent to that of the mother (277), and occurs in small quantities in the spinal and other body fluids of adults (64), (238).

In most vertebrates other than man the blood uric acid is exceedingly small. Folin and Denis (81) report values of 0.05 to 0.2 mgm. per 100 cc. in the rabbit, sheep, pig, horse, monkey, ox and cat. Birds, in whom uric acid is the end-product of protein metabolism, are exceptions to the rule of low blood uric acid. The chicken, duck and goose normally have about 4.8 mgm. per 100 cc. (81), (31). In the blood of fishes uric acid also occurs in rather large amounts (58). Teleosts contain about 4 mgm., and elasmobranchs approximately 1 mgm. per 100 cc. The blood of arthropods shows 0.3 to 3.8 mgm. per cent (233).

Of the abnormal variations in the uric acid content of human blood, the most interesting occur in nephritis and gout. Folin and Denis (83) showed that marked increases occur in uremia. At about the same time, Myers and Fine (237) reported values as high as 27 mgm. per 100 cc. of blood in terminal interstitial nephritis. They also pointed out that in 6 cases of gout the uric acid values varied between 3.8 and 5.8 mgm. The marked retention in nephritis leads to a rather even distribution of uric acid in the tissues (64), (318). It is not remarkable that uric acid retention should occur during deficient renal function, but the important fact originally pointed out by Myers, Fine and Lough (239) is that usually uric acid is the *first* nitrogenous waste product to be retained. In incipient interstitial nephritis, before the urea or creatinine show appreciable increases, values of 6 or more milligrams per cent of uric acid may be found. This observation has been verified by many others, notably Baumann, Hansmann, Davis and Stevens (27), who regard the determination of the uric acid concentration in the blood as one of the most delicate indexes of renal function available.

In gout, uncomplicated by nephritis, the uric acid is increased (66), (84), (253), (65), (92), and usually ranges from 4 to 10 mgm. per 100 cc.,

while the values for the urea and creatinine remain practically normal. The blood picture, therefore, is very similar to that in incipient nephritis. Indeed, the similarity is so striking that Fine (65) has raised the question as to whether gout is not a very early manifestation of nephritis, which may or may not further develop. Of interest in this connection are the findings of McClure (215). This author obtained evidence of renal insufficiency in each of six cases of gout, although no clinical symptoms of nephritis were displayed.

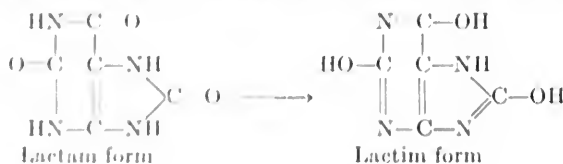
Certain drugs, notably salicylates (68), (55), and 2-phenylquinoline-4 carboxylic acid (atophan) (85), (217), (66), (279) greatly increase the urinary excretion, and lower the blood concentration of uric acid in gout. The increased elimination, however, practically ceases in two days, and more prolonged use of the drug fails to induce further decrease in blood uric acid (66). Probably the drugs act by increasing renal permeability, and perhaps also by accelerating mobilization of uric acid from the tissues (96). In advanced nephritis, atophan has slight or no effect upon uric acid excretion (67). Sodium benzoate has also been said to increase the output, and to lower the concentration in the blood (56), but according to Lewis and Karr (202) its administration may actually lead to decreased elimination.

In addition to gout and nephritis, in which the difficulty is obviously one of renal inefficiency, the uric acid content of blood may be markedly increased in conditions associated with an exaggerated nuclear catabolism. Leucemia (236) is a case in question. Under such circumstances the kidneys may excrete large amounts of uric acid, but apparently are unable to keep pace with its formation. Although in the normal individual there is no threshold concentration for uric acid excretion, such as exists in the case of sugar, nevertheless the kidneys appear to dispose of an overload very slowly. Probably this is not due to the relative insolubility of urates, for the blood (291), and usually the urine (105), are able to dissolve at body temperature more uric acid than they are called upon to handle. More likely the lags in excretion, with resulting accumulation in the blood, are associated with the fact, pointed out by Myers and Fine (238), that the kidneys normally are able to concentrate uric acid only about 20 times, while they concentrate urea and creatinine 80 and 100 times respectively.

Other illustrative conditions in which the uric acid of the blood may be increased are pneumonia (83), (146), malignancy (147), arthritis (84), lead and mercury poisoning (236), methanol poisoning (255), and toxemias of pregnancy (333). Curiously, a marked increase has recently been reported in polyneuritis of chickens (304).

As regards the distribution between the plasma and the corpuscles in human blood, uric acid manifests peculiarities which at the present time are impossible of explanation. Of 20 samples of blood examined, Bornstein and Griesbach (46) found 2 with equal distribution, on a volume basis, between the cells and the plasma, 13 in which the uric acid was greater in the cells, and 5 in which it was greater in the plasma. They believe that in shed blood two enzymatic processes occur, one tending to increase, and the other tending to decrease the quantity of uric acid, and that these reactions occur exclusively in the blood cells. In 104 distributional analyses, Theis and Benedict (303) found 51 samples of blood with equal distribution, 8 with greater concentration in the cells, and 45 with larger amounts in the plasma. This relationship was independent of the pathological condition. On adding uric acid to 20 different bloods, these authors found an equal distribution in 30 per cent of the cases, while in 70 per cent the added uric acid did not penetrate the cells at all. They point out that these marked differences in permeability may occur possibly in other cells of the body as well as in the blood corpuscles, and "may tend to throw light on the questions involved in specific uric acid retention in the organism." In analyses of 20 normal bloods, Wu (336) uniformly found about twice as much uric acid in the plasma as in the corpuscles. More recently Plass (250) has reported that in acute retention uric acid accumulates in the plasma first, but later diffuses into the blood cells until the concentration in the latter is practically equal to that in the plasma. Following relief from such a retention, the corpuscles may actually contain more uric acid than the plasma, due to the fact that excretion occurs more speedily than diffusion from the cells into the plasma. It is difficult to harmonize these four observations, and further experiments of a similar nature are needed. It appears not improbable that illuminating results might follow intensive investigations of this sort.

At various times the suggestion has been made that blood may contain more than one form of uric acid. The discovery by Gudzent (97) that uric acid is capable of keto-enol tautomerism, immediately suggested the possibility that different proportions of the so-called *lactam* and *lactim* forms might occur in normal and abnormal bloods. Gudzent believes that only the monosodium salt of the more stable but



less soluble lactim form can occur in blood. This idea would appear to be negatived by the finding of amounts of uric acid far in excess of the solubility of the lactim form, which according to Gudzent is only 8.3 mgm. per 100 cc. of serum. One may assume with Bechhold and Ziegler (28) that part of the urate is present in a colloidal condition, but definite proof of this assumption has not been adduced. Of extraordinary interest, therefore, is the discovery of Benedict (31) that ox blood contains a d-ribose-uric acid compound, which he and his associates have succeeded in isolating (54), and which along with the free uric acid, occurs exclusively in the erythrocytes (245). The relatively large amount of this riboside (?) present is indicated by the fact that while fresh ox blood contains only about 0.5 mgm. of free uric acid per 100 cc., 7.5 mgm. may be found after hydrolysis with hydrochloric acid. The uric acid is also liberated by enzymatic action, when the blood is allowed to stand for several days under aseptic conditions.

Continuing the work begun in Benedict's laboratory, Newton and Davis (246) have obtained evidence of the existence of combined uric acid in human, horse, sheep, pig, dog and chicken blood. The quantity in ox blood far exceeds that in the blood of any other animal examined. Human blood appears to be next to ox blood in its combined uric acid content, but only minute traces are detectable in the other species. An interesting distributional difference in the uric acid of ox and chicken bloods was observed by Benedict (31). In contrast to the fact that uric acid in the former is confined to the erythrocytes, in the latter it is almost wholly in the serum.

The ribose-uric acid compound is an exceedingly stable one, and until hydrolyzed does not react with the reagents used in Benedict's colorimetric method. Recently Morris and Macleod (235) have reported the occurrence in certain specimens of human blood of a form of uric acid dimorphism, which they regard as quite distinct from that of Benedict. The evidence which they present is as follows: *a*, The Morris-Macleod method (334) for uric acid estimation gives higher values in some specimens of blood than the procedure of Folin and Wu (87). *b*, From mixed samples of human blood more uric acid can be isolated in crystalline form than is indicated by the Folin-Wu determination. *c*, The addition of excess oxalate to blood samples tends to increase the values obtained by the Folin-Wu method, until they approximate those by the Morris-Macleod procedure. In the writer's judgment the above facts do not prove that blood uric acid is dimorphous in the sense of Morris and Macleod, but are merely *suggestive* of such an occurrence. In any

event, investigations along this line are exceedingly promising. It seems not unlikely that the defect in the metabolism of purines in gout eventually will prove to be associated with either an abnormal distribution, or an unusual form of uric acid in the blood.

7. THE EFFECT OF PURINE-FREE FOODS UPON THE OUTPUT OF ENDOGENOUS URIC ACID. Abundant evidence has been accumulated in recent years to indicate that the original idea of Burian and Schur (51) and Sivén (276), to the effect that the excretion of endogenous purines is constant from day to day in the same individual, is not correct. More than 18 years ago Folin (78) found that the change from a diet of milk and eggs to one of starch and cream might be accompanied by a fall in uric acid elimination of practically 50 per cent, although both diets are purine-free.

Since the work of Folin, numerous investigators, notably Leathes (174), Mendel and Brown (220), Smetánka (278), Taylor and Rose (294), Mendel and Stehle (223), Lewis and Doisy (200), Höst (113) and Rose (262) have corroborated his findings that protein ingestion exerts a marked influence upon urinary uric acid. Smetánka (278), Mendel and Stehle (223), and Umeda (308) also observed an increase in uric acid elimination, as compared with the fasting output, following the ingestion of carbohydrates. Recently Lewis and Corley (199) have reported similar effects from the consumption of honey and glucose syrup in amounts greater than 200 grams, but observed no effects from moderate amounts (100 grams) of glucose, sucrose or lactose. Apparently fats produce the least effect upon purine metabolism of either of the foodstuffs.

The calorific value of the diet is likewise important in determining the uric acid excretion. According to Graham and Poulton (95), diets of protein and fat of insufficient caloric value cause a fall of 30 to 50 per cent in the output of endogenous uric acid. If most of the fat is replaced by carbohydrate no fall is observed. Höst (113) has affirmed that every increase or decrease in the calorific value of the food beyond a certain minimum, is accompanied by a like change in uric acid excretion. Rose (262) also observed distinct increases in uric acid excretion in passing from a low calorific to a high calorific ration, while maintaining the protein intake at a constant level.

No unanimity of opinion exists, however, as to the cause of the increases and decreases in endogenous uric acid elimination induced by changes in the diet. Of the many possible theories offered in explanation of the observed phenomena, the following require brief consideration.

*a. Nuclear disintegration occasioned by the work of digestion and food storage.* This theory was first suggested by Mareš (214), and was later modified by Smetánka (278). Mareš attributed the increase in output of uric acid following the consumption of purine-free food to nuclear disintegration, chiefly in the alimentary glands, incidental to the physiological work of secretion and digestion. According to this author, uric acid represents the wear and tear of glandular tissues. As evidence for this mechanism, Mareš points not only to the effect of foods, but to the fact that pilocarpine, which is known to increase secretory activity, likewise augments the excretion of uric acid. Lambling and Dubois (173), and Höst (113) also regard digestive work as an important factor in the variations in output of endogenous uric acid, while Mendel and Stehle (223) state that their experiments "offer no obstacle to the assumption that a portion, at least, of the endogenous uric acid may originate from the activity of the alimentary secretory apparatus."

Smetánka, who in the main adheres to the Mareš theory, was forced to modify it. Having observed that the ingestion of honey, a food which requires practically no digestion, causes a marked increase in the output of uric acid, this investigator suggested that in addition to digestive work, the activity involved in glycogenesis may be responsible for a part of the endogenous uric acid. On the other hand, we believe the Mareš-Smetánka theory may be definitely excluded from serious consideration in view of the work of Lewis, Dunn and Doisy (201). These authors showed that glycocoll, alanine, and other amino-acids, which obviously require no digestion, and which in the amounts given could not have formed appreciable quantities of glycogen, increase the hourly output of uric acid as much as do proteins.

*b. Stimulated purine synthesis.* The well-established fact, considered in some detail above, that the animal organism can synthesize purines in so far as they are required for anabolic purposes, suggests the possibility that these synthetic processes may be stimulated by food ingestion. Indeed Graham and Poulton (95) and Umeda (308) have suggested that part of the endogenous uric acid may arise through synthesis from carbohydrates. They observed that carbohydrate-rich fat-poor diets cause a greater excretion of uric acid than do fat-rich carbohydrate-poor diets, even though the protein content and calorific value of the food are maintained constant. Umeda suggests that uric acid may arise from a condensation of urea with an intermediary product of carbohydrate metabolism, perhaps lactic acid. Graham and Poulton point to the observation of Knoop and Windaus (150), that when glucose is exposed

*in vitro* to the action of sunlight and the strongly dissociated compound,  $\text{Zn}(\text{OH})_2 \cdot 4\text{NH}_3$ , methyl glyoxal and 5-methyl-imidazole are formed. As interesting as these suggestions are, there exists at the present time no experimental evidence *in vivo* which justifies the belief that carbohydrates are transformed into purines in the animal organism.

On the other hand, the possibility of a stimulating effect of proteins upon purine synthesis cannot be excluded so easily. In this connection the work of Ackroyd and Hopkins (10) already referred to, is of special interest. As stated above (p. 560), these authors believe that either arginine or histidine may serve as the substrate for purine formation. A similar conclusion as to the origin of purines in the diamino-acids was arrived at by Harding and Young (104). According to these investigators, the feeding of placenta, which has a high content of arginine, causes a much greater increase in the output of uric acid and allantoin in young dogs than does the ingestion of an equal quantity of muscle protein.

On the contrary, Abderhalden and Einbeck (2), Abderhalden, Einbeck and Schmid (3), and Lewis and Doisy (200) were unable to show any relationship between the diamino-acid content of the diet and the uric acid or allantoin output in the urine. Lewis and Doisy compared the effects of diets high and low in arginine and histidine upon the uric acid output in man. Abderhalden and Einbeck studied the effects of adding histidine to the diet upon the allantoin excretion in the dog. In the later experiment of Abderhalden and his co-workers (3), histidine hydrochloride was given in 10 gram doses to a fasting animal. Neither of the experiments yielded any indication of purine formation from the diamino-acids.

In contrast to the methods of Abderhalden and Lewis and their co-workers, Ackroyd and Hopkins compared the effects of diets *free* from arginine and histidine, with diets containing *adequate amounts* of the diamino-acids. This difference in experimental method is an exceedingly important one. For even if it be admitted that tissue purines have their ultimate origin in arginine and histidine, this fact does not warrant the assumption that the extent of purine synthesis is proportional to the arginine-histidine supply. On the contrary, it seems reasonable to suppose that the synthesis of a tissue component is limited quantitatively to the anabolic needs of the organism for that particular ingredient. As soon as a diet contains sufficient precursors of a given anabolite, synthesis probably occurs at the optimum rate. We believe that this view is entirely in accord with that of Ackroyd and Hopkins. If this

conception is correct, one should not expect an increase in uric acid excretion to follow the feeding of purine precursors, unless the preceding diet were deficient in these precursors. It therefore seems unreasonable to assume a stimulated formation of purines following food ingestion, unless a second assumption is also made, namely, that part of the uric acid in man as in birds is an *end-product of protein catabolism*. We know of no experiments which justify the second proposition.

*c. Stimulation of cellular metabolism.* In the interesting investigation of Lewis, Dunn and Doisy (201) on the influence of diet upon the hourly elimination of uric acid, the authors suggest that the effect of the ingestion of protein or amino-acids may be due to a general stimulation of cellular metabolism. Each of the four amino-acids, glycocoll, alanine, glutaminic acid, and aspartic acid, as well as the closely related asparagine, caused an appreciable increase in the hourly fasting output of uric acid. The stimulation caused by the dicarboxylic amino-acids was more marked than that produced by glycocoll and alanine. On the other hand, sarcosine, a substituted amino-acid not readily catabolized by the body, and ammonium chloride and urea, were without influence.<sup>4</sup>

For reasons which have been discussed in detail elsewhere (261), we believe that the cellular stimulation theory is the most satisfactory one available at the present time. We (262) have shown that following radical increases in food consumption (particularly as regards the protein portion), the largest alterations in uric acid output occur on the days immediately succeeding the dietary changes. These observations led us to believe that the amino-acids themselves, rather than their intermediary catabolic products, are responsible for the stimulating effect upon cellular activity. On the other hand, the recent findings of Gibson and Doisy (93), that pyruvic acid causes an increase in the hourly excretion of uric acid, and of Lewis and Corley (199) that glycerol exerts a similar effect, indicate that the native foodstuffs and amino-acids are not the only substances which increase uric acid elimination. Moreover, the situation is further complicated by the fact that while pyruvic acid causes an increase, the closely related lactic acid occasions a decrease in uric acid output (93).

<sup>4</sup>Since writing the above, A. A. Christman and H. B. Lewis (Journ. Biol. Chem., 1923, lvii, 379) have reported that the administration of amino-acids in rabbits is followed by *decreases* in allantoin excretion. These observations are in marked contrast to those observed in human subjects, and suggest a possible difference in physiological significance of uric acid in man and of allantoin in lower animals.

It is not likely that in the near future our knowledge will be sufficiently extensive to enable us to accurately picture the details of endogenous purine metabolism, or to fully explain the action of purine-free substances upon uric acid excretion. The problem is a difficult one, and the interpretation of the experimental findings requires unusual caution. But until evidence to the contrary is obtained, the cellular stimulation theory may serve as a helpful working hypothesis.

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## THE MODERN THEORY OF GENETICS AND THE PROBLEM OF EMBRYONIC DEVELOPMENT

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The several attempts that have been made in the past to "explain" the development of the individual from the egg by postulating physiological units, pangenes, determinants and other kinds of representative particles have, not without reason, called forth a good deal of criticism as to the value of such a procedure, especially in the biological sciences where the evidence for an assumption of this kind was largely wanting.

The disrepute into which these philosophical speculations fell has carried over, and there has remained, in certain quarters at least, a prejudice against the modern theory of heredity, based on Mendel's laws, that also postulates units or elements in the germinal materials. A little discrimination might have shown that because certain speculations concerning developmental units had little evidence in their support, and were objectionable on more than one score, it did not follow that the egg and sperm might not contain discrete elements, chemical compounds, through whose perpetuity heredity is possible and through whose effects the process of embryonic development might be influenced. It was scarcely rational to reject all theories assuming the germ-cell to contain a number of definite substances that are the agents through which heredity takes place, because a few philosophers had postulated fictitious units. The value of any hypothesis relating to heredity and to development will depend of course on the kind of evidence that can be brought to its support.

Without attempting to review the history of the theories of representative particles that have played a rôle in modern biological speculations, I shall first point out the kind of evidence on which our interpretation of embryonic development rests and then give the evidence from genetics that leads to the conclusion that heredity is due to discrete particles in the germinal material. If I can succeed in removing some of the unnecessary prejudices that seem still to persist on the part of those not familiar at first hand with the evidence, I shall have

accomplished the purpose of this article. While it is not possible to bring forward here more than a fraction of the evidence on which the modern theory of heredity rests, it should be possible, even in a limited space, to indicate the kind of evidence that supports this theory.

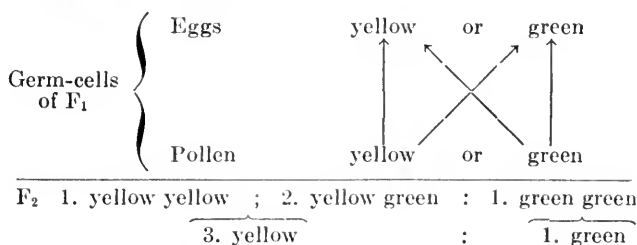
The assumptions that underlie our modern ideas as to the relation between genetics and development are the following: *a.* The orderliness of the developmental process is a fact of observation. The eggs of an individual develop into the same kinds of individuals, if the environment is constant. *b.* Since vastly different kinds of animals (species) develop in the same environment, in the sea, or in a lake, for instance, their differences must depend on differences in the eggs themselves. *c.* If an alteration takes place in the composition of an egg, the end product of its development is expected to be different from what it was before, and if this alteration should be present again in the eggs of the next generation, the same effects are expected. An alteration of this kind is called a mutation. These generalizations rest on actual experience and, as will probably be conceded by everyone familiar with the evidence, they mean no more than that the developmental process is regarded as a causal phenomenon, or in other words, it appears to be an orderly sequence of recurrent events.

When, however, we come to enquire more specifically into the details on which these generalities are based, we find that we have not penetrated very far into the nature of the changes that are postulated. Nevertheless it can be shown, I think, that there are sufficient grounds for holding such views.

The evidence for the statement that at times a change may take place in an egg (or sperm cell) of such a sort that it persists in the eggs (and sperm cells) of succeeding generations, is derived from new types that suddenly appear and reappear in the descendants of such mutant types. Before bringing forward this evidence, there is a further relation that should be considered. It is this. Between the egg and the end product of its development—the individual—we are familiar with a long series of events as a consequence of which a specific form emerges. While there is an extensive literature describing these stages, it is generally admitted that we know very little as to the nature of the changes that take place. Our idea that they are causal events rests largely, as I have said, on their orderliness, which is the same for countless individual cases. Are we justified, then, in postulating that a change in the germ-cell has occurred whenever a new type appears that is, in a sense, permanent? Since every individual starts as an

egg, there seems to be no escape from this interpretation, which is moreover in accord with a vast body of information showing that the egg of an individual is a cell that has been derived by successive generations from a similar cell (the egg) of the preceding generation.

The evidence that something in the germ-material that is correlated with typical characters perpetuates itself, is derived from Mendel's law of segregation. This may be illustrated as follows: If a yellow pea, ( $P_1$ ), is crossed to a green pea, ( $P_1$ ) the peas produced by the cross ( $F_1$ ) are yellow. If these are self-fertilized, their offspring,  $F_2$ , consists of yellow and green peas in the ratio of three yellows to one green. When these,  $F_2$ , in turn are tested, it is found that the green peas breed true to green, and that there are two kinds of yellows in the ratio of two to one. The former, if selfed, produce again yellow and green peas in the same ratio of 3:1, while the latter, if selfed, breed true to yellow. Mendel pointed out that these numerical results can be explained by a very simple assumption. The original yellow pea,  $P_1$ , carries something in its germ-cells that makes it yellow, and the green pea, something in its germ-cells that makes it green. When the two are brought together in the first generation hybrid,  $F_1$ , the result is not a blend of the two, but the yellow dominates. (This, however, is of no importance, for in many other cases the hybrid is intermediate.) If when the germ-cells of the hybrid come to maturity, the two somethings (that made one type yellow and the other type green) separate (segregate), then half of the germ cells will be yellow-producing and half will be green-producing.



Chance fertilization of any egg by any pollen grain will give three kinds of combinations, namely, 1, yellow yellow; 2, yellow green; 3, green green. Of these three combinations the first will be pure for yellow, the second (which are twice as numerous) will be mixed yellow and green in composition, but yellow in expression, since, as shown in the first generation, when both are present the yellow dominates; the third will be pure for green. These classes are in the ratio of 1:2:1.

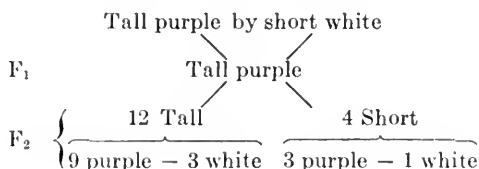
Mendel's theory rests, therefore, on the assumption that if in the germ-cells of the hybrid the two parental contributions—the yellow-producing and the green-producing contributions—separate (segregate) so that half of the germ-cells are of one kind and half of the other, and if the chances are equal that any pollen grain may fertilize any egg, the observed numerical results in the second generation are accounted for

A simple experiment of this kind reveals nothing as to the nature of the germinal material of the two parents. For instance, it would not be necessary to assume that there is an element for yellow and one for green, although obviously this would be the commonplace way of stating the relation; for when, as here, the assumption of a difference between the two kinds of peas suffices for the theory, there is nothing to demonstrate that there must be representative units in the germ material that stand for yellow or green. The explanation would be exactly the same if it were stated that all that went into the hybrid (from the yellow parent) separated from all that went into the hybrid (from the green parent). But this interpretation will not cover all the results found by Mendel, as is made clear by those cases where two or more character-differences are found in the same cross. This evidence is furnished by Mendel's second law, or law of independent assortment of the genes. It may also be illustrated by an example.

Tall and dwarf peas form a Mendelian pair of characters, i.e., when crossed they give in  $F_2$  a 3 to 1 ratio, etc. Purple flower color and white flower color are another pair of such characters. If a pea that is tall and has purple flowers is crossed to a pea that is short and has white flowers, the offspring,  $F_1$ , will be tall with purple flowers. If these tall purple hybrids self-fertilize, their offspring,  $F_2$ , will fall into four classes, namely, tall purple, tall white, short purple, and short white, in the ratios of 9:3:3:1.

Eggs		T P	t P	T p	t p
Pollen	T P	T P T P tall purple	t P T P tall purple	T p T P tall purple	t p T P tall purple
	t P	T P t P tall purple	t P t P short purple	T p t P tall purple	t p t P short purple
	T p	T P T p tall purple	t P T p tall purple	T p T p tall white	t p T p tall white
	t p	T P t p tall purple	t P t p short purple	T p t p tall white	t p t p short white

It is obvious that the result can not be explained, as in the case where a single pair of characters only is involved, by assuming that the whole germ material of the original tall purple parent separates in the germ-track of the hybrid from the germ material of the short white parent; but it is equally obvious that, as Mendel pointed out, the results can be explained if we treat each pair of characters that entered the cross as though it behaved independently of the other pair of characters. For example, if the  $F_2$  offspring are separated into tall and short, they will be as 3 to 1. Amongst the tall again, there should be purples and whites in the ratio of 3 purple and 1 white, and among the short there should also be found three purples to 1 white. Since there are three times as many tall as short, the expectation for all the offspring is 9 tall purple, 3 tall white, 3 short purple, and 1 short white. The following diagram represents these relations.



Mendel also found that when three pairs of characters enter a cross, each pair behaves independently of the other two pairs, or, stated more accurately, the numerical results obtained, when three pairs of characters are present, can be accounted for, if each pair of characters segregates independently of the segregation of the other two pairs.

Mendel's second law has been shown to be limited in its application. A new phenomenon, known as linkage, places serious restrictions on the generality of the law of independent assortment. Mendel did not meet with any cases of linkage, or if he did he has left no statements in regard to them. In recent years many such cases have been found, including a few in garden peas. However, the free assortment shown by two pairs of characters is proof that the germ-materials that went in and segregated in the hybrid are not wholes, but must be treated as separable into parts. How far this material may be subdivided can only be determined at present by the genetic evidence.

The evidence from free assortment carries us only a short distance in the analysis of the divisibility of the germ-materials. It has led, however, in recent years to a very important conclusion, namely, that the number of independent factor-pairs corresponds with the

number of the chromosome pairs. In other words, the evidence from Mendel's second law has shown that the divisibility of the hereditary material extends as far as the chromosomes. But to-day we go far beyond this conclusion, and we are able to do so because there has been discovered another phenomenon, crossing-over, that enables us to work within the linkage groups.

*Linkage and crossing-over.* The evidence from genetics shows that in many cases when two characters are present in the same cross they do not freely assort, but the characters that went in from the one or from the other parent tend to remain together in subsequent generations. If they always remain together they are said to be completely linked; when they separate occasionally, they may be said to be closely linked; when they separate more freely, they are loosely linked. A few examples will bring out these relations more clearly.

When the sex-linked characters of *Drosophila*, yellow wings and white eyes, go in together on one side, and gray wings and red eyes on the other, 98.5 per cent of the  $F_2$  flies are either yellow white or gray red. Only 1.5 per cent of the  $F_2$  flies are cross-overs, i.e., yellow red or gray white (fig. 1). Here the linkage is close.

When yellow wings and vermilion eyes enter from one side and gray wings and red eyes from the other side, 69 per cent of the  $F_2$  offspring are the classes that went into the cross together, namely, yellow vermilion and gray red, and 31 per cent of the  $F_2$  flies are cross-overs, namely, yellow red and gray vermilion. Here the linkage is not so close.

When yellow wings and bar eyes enter from one side and gray wings and round eyes from the other side, 44 per cent of the  $F_2$  offspring are the classes that went in together, namely, yellow round and gray bar. This is a case of loose linkage. Similar examples could be given from each of the other three linkage groups of *Drosophila*.

These facts do not reveal one of the most fundamental discoveries relating to linkage, which is apparent only when representatives of a sufficiently large number of linked characters enter the cross at the same time to enable us to detect the nature of the process that is taking place. When such an experiment is made, the results show for a given linkage series, such, for example, as the sex-linked series of *Drosophila*, that no crossing-over at all occurs in 43 per cent of the eggs, and that crossing-over occurs once in about 43.5 per cent of the eggs.<sup>1</sup>

<sup>1</sup> Crossing over occurs twice in 13 per cent of the eggs and three times in 0.5 per cent

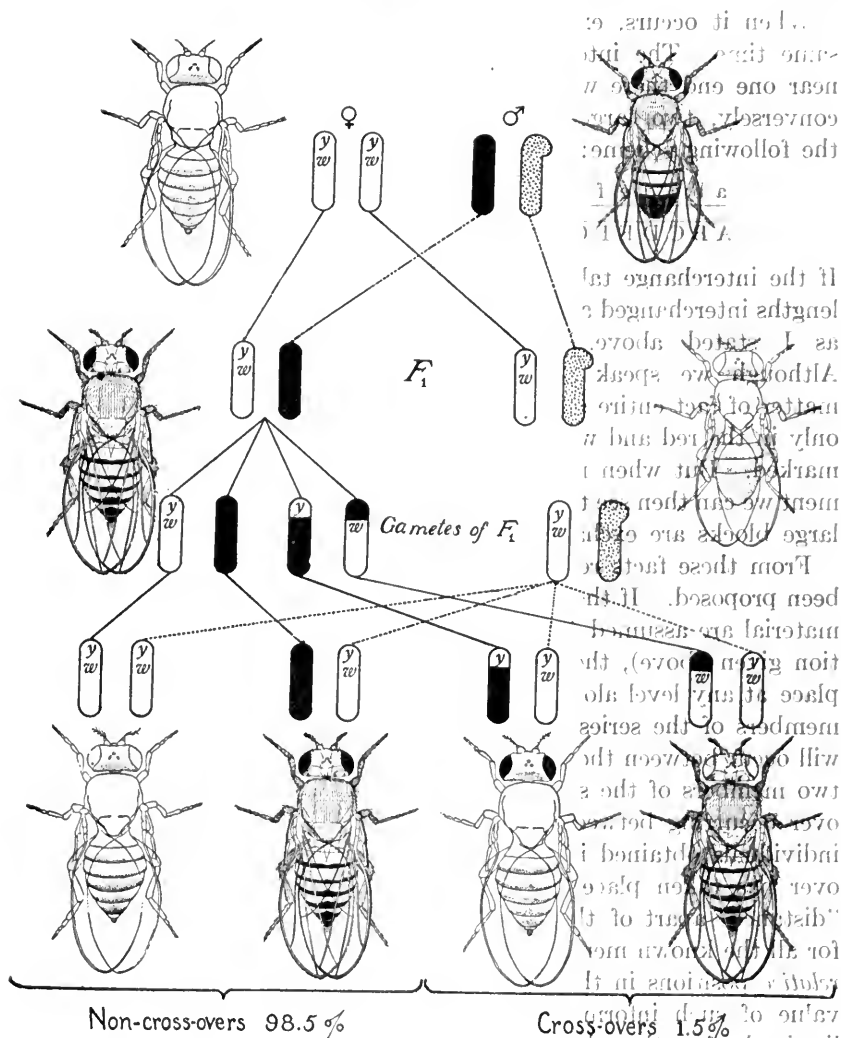


Fig. 1. Cross of a female *Drosophila melanogaster* that has white eyes and yellow wings to a wild type male with red eyes and gray wings. The F<sub>1</sub> daughters are like their mother and the F<sub>1</sub> sons are like the father. In the lower line of the diagram the four kinds of F<sub>2</sub> flies are shown. The first two are like their grand parents and represent non-cross-over classes. The other two are the cross-over flies.

When it occurs, extensive series of genes are interchanged at the same time. The interchange may occur at any level. If it occurs near one end there will be two short blocks interchanged, (or stated conversely, two large blocks interchanged). This is represented in the following scheme:

$$\begin{array}{c} \underline{a\ b\ c\ d\ e\ f\ g\ h\ i\ j\ k\ l\ m} \quad \underline{A\ B\ C\ d\ e\ f\ g\ h\ i\ j\ k\ l\ m} \\ \underline{A\ B\ C\ D\ E\ F\ G\ H\ I\ J\ K\ L\ M} \quad \underline{a\ b\ c\ D\ E\ F\ G\ H\ I\ J\ K\ L\ M} \end{array}$$

If the interchange takes place near or at the middle of the series the lengths interchanged are the same, etc. Results of this kind illustrate, as I stated above, an important fact concerning crossing-over. Although we speak of red and white eyes interchanging, as a matter of fact entire series of genes cross-over, but the event is visible only in the red and white because this is the pair that is, so to speak, marked. But when many characters are present in the same experiment we can then see that whenever red and white eyes are interchanged large blocks are exchanged with them.

From these facts relating to crossing-over, the following theory has been proposed. If the genes, that represent the characters in the germ-material are assumed to be arranged in linear order (as in the illustration given above), then, if crossing over is an event that may take place at any level along the chromosome's length, it follows that if two members of the series lie near together the chance that crossing-over will occur between them is small; and conversely the further apart any two members of the series lie, the greater the probability of crossing-over occurring between them. Now, since the number of cross-over individuals obtained in any case is a measure of how often crossing-over has taken place, we can calculate from such data the *relative* "distance" apart of the two factor pairs in question. If this is done for all the known members of any series we can get an idea as to their *relative* positions in the linear series with respect to each other. The value of such information, aside from its great theoretical interest, lies in the fact that these assigned numerical values allow us to predict how a new member of any series will behave with respect to all the others of that series, provided its numerical relation to two members of the known series is first determined. This is done by testing its crossing-over with any two members of the series, preferably two that are not too "distant." The ability to predict new combinations is one of the ear-marks of a scientific hypothesis, and in this respect the theory of the linear order of the genes fulfils the requirements.

Instead of trying to justify the theory on these grounds, there is a logical or geometrical demonstration of the linear order of the genes. For example, yellow wings and white eyes give a cross-over value of 1.2 per cent, white eyes and bifid wings give a cross-over value of 3.5 per cent. Now if the order is yellow white bifid, then yellow and bifid, if tested, are expected to give a cross-over value that is the sum of the other two; but if the order is white yellow bifid, then the yellow bifid cross-over value is expected to be the difference between white bifid and yellow bifid. The data show that the former situation obtains in this case.

This relation for three or more points holds in all cases so far carefully investigated with the proper controls. It may be stated in a general way, as follows: The cross-over values of three characters *a*, *b*, *c*, are such that the cross-over value of *a* and *b* is always found to be the sum or the difference of the cross-over values of *a* and *c* and that of *b* and *c*. This relation is a geometric relation for three points in a line. No other simple relation fulfils these conditions.

It is necessary to emphasize that these conclusions are derived entirely from genetic data, because the theory of crossing over in its further development has become involved in more hypothetical inferences relating to the chromosomes. The full significance of the genetic theory would remain even if the chromosomal inferences turned out to be quite different from those proposed. Nevertheless, I think, that it is a matter of great interest to find in the known behavior of the chromosomes certain stages that fulfil all the requirements of the genetic theory.

*Double crossing-over.* There is genetic evidence that crossing-over may take place twice in the same series. Inasmuch as double crossing-over, as it is called, introduces the necessity of a correction in the data when only two pairs of linked characters are studied, it is important to make clear how this process affects the results. Again an example will serve our purpose: Suppose an individual with the three sex-linked factors, white eyes, miniature wings and forked bristles, is crossed to an individual with the normal allelomorphs of these characters, red eyes, long wings and straight bristles. In the hybrid female the two X-chromosomes bearing the genes for these characters may be represented as follows:

white	miniature	forked
red	long	straight

Now if crossing-over should take place at two levels between white and forked, the relation of the three pairs of genes involved would then be represented as follows:

white	long	forked
red	miniature	straight

If the cross-over value of white-red and forked-straight were alone being studied, the combinations of these characters that come out after such a double crossing-over would be the same as those that went in, namely, white-forked and red-straight as shown in the second diagram above. Consequently, these individuals would be classified as non-cross-overs and two crossings-over would be missing. This would give relatively too low a crossing-over value for this combination in comparison with cases where no double crossing-over occurs, and the percentage values would be imperfect to the extent to which double crossing-over had occurred.

If, however, in such a cross a third character is taken into account—one which previous experiments had shown to be between the extremes—then double crossing-over will be detected when it occurs, for the double crossing-over will give flies that are white long forked and red miniature straight (see diagram).

Experience has shown that when the cross-over value of two factor-pairs is small, no double crossing-over occurs, and that the chance of its occurring increases as the cross-over values become larger. This relation may be stated in a simple way; namely, that when crossing-over takes place at one level the region on each side is protected from a second cross-over in the same egg.

The further away any level lies, the greater is the likelihood of double crossing-over taking place, at least up to a certain distance. It is true, of course, on chance alone, the greater the length of the series the greater the expectation of a second crossing-over occurring, but the observed relations show that chance alone will not account for the facts. The only explanation so far formed involves a consideration of the chromosome behavior and will be described below.

If the chance of a second crossing-over steadily increases as the distance from the first crossing-over increases, a level might be reached which is no longer affected by the first crossing-over. This relation has also been found, by Weinstein, to hold. The evidence for this is important for any consideration dealing with the mechanism of crossing-over; for, whatever hypothesis of crossing-over is suggested, it

must fulfil the requirement of these discovered relations. There is, in fact, a mechanical factor which will account for this relation. If the interchange takes place at the time of conjugation as a result of the twisting or looping of the pair of chromosomes involved, the semi-rigidity of the thread imposes such conditions on the situation.

It will have been observed that cross-over values have been often spoken of as though they could be converted into distance apart of the genes. If understood properly, the use of the more graphic term distance should not lead to confusion, but, since it has been misunderstood even by geneticists, a word of caution may not be superfluous. An imaginary situation will serve to bring out the most essential point. If in one stock, crossing-over should be more frequent between two factor-pairs than between the same factor pairs in another stock the "distance" would appear to be greater in the former. Obviously, under such circumstances distance could not have the precise meaning that it appears to have when cross-over values are converted into distance. Only when the cross-over values are constant could we compare two independently obtained results. Now, as a matter of fact, cross-over values are remarkably constant under known conditions. On the other hand there is abundant evidence to show that crossing-over may be influenced both by external and by internal factors. These can be studied by experimental methods and the corrections necessary to convert one set of observations into another can be obtained. Therefore, in making out tables or "maps" of the linkage groups, it is expedient to exclude those cases where unusual environmental or genetic conditions have been found to be present. The numerical values assigned to the series of linked genes in our maps represent what may be called normal values, i.e., those most frequently met with when known disturbing factors are eliminated.

Distance as a genetic term is, after all, not the most interesting fact brought out by this work in crossing-over, even although the practical aspects of assigning values to the position of the genes in the series are manifest. It is the linear order of the genes and their retention of this order through all the vicissitudes of changes in crossing-over that is the most significant feature of the results. A striking case in point is that studied by Sturtevant. He found that there is a region at the right end of the chromosome extending for a distance of 56 units that is affected by a genetic factor in such a way that crossing-over is cut down almost to zero, but the little crossing-over that remains shows that the order of the genes is not changed.

A recent "map" of the four linkage groups of *Drosophila melanogaster* is shown in figure 2. Only the more familiar or more used genes are named, the other cross lines indicate the known location of still other genes. In addition to these there are as many more characters known, but they are not so accurately placed as are those here shown. In making these maps the linear order of the series is determined from material as homogeneous as possible, i.e., from pair cultures under favorable environmental conditions and where no known internal disturbing factors are present. The linear order once determined in this way, the cross-over values assigned to the loci are the average results of a great amount of data, often obtained at various times and from various sources, excluding all those cases known to be exceptional. Values obtained in this way give the best approximations to those most likely to be met with.

There is also some evidence to show that crossing-over in certain regions of the linkage group is more likely to occur than in other regions. This disturbs the relative values of the distances, but since this is a constant variant, it does not call for a correction in the maps. For the further working out of the problems of crossing-over, however, the regional difference may be highly important.

*The chromosome mechanism.* The evidence that the chromosomes are intimately concerned with the inheritance of Mendelian characters has steadily grown more convincing. The fact that the behavior of the chromosomes at the reduction period furnishes a mechanism that parallels the postulates of Mendel's two laws in all respects, would in itself attract the immediate attention of any biologist who was not content to have these laws stand without regard to processes taking place in the eggs and sperm cells. Even were there no other evidence, I think that biologists would have welcomed this application of Mendelian principles to cytology, but there is at present other, direct evidence pointing to the chromosomes as the bearers of the Mendelian genes. The correspondence of the linkage groups in *Drosophila melanogaster* is important in this connection, for if the chromosomes carry the genes, and if they are, as they appear to be, self-perpetuating bodies in the cell, such a phenomenon as that of linkage is expected. Linkage, in this sense, means only that the chromosome remains a constant element from one generation to the next. What was not to be anticipated on such a view, however, was the genetic discovery that this constancy of the chromosomes holds only as far as other chromosomes are concerned. It does not hold for members of the same pair. Be-

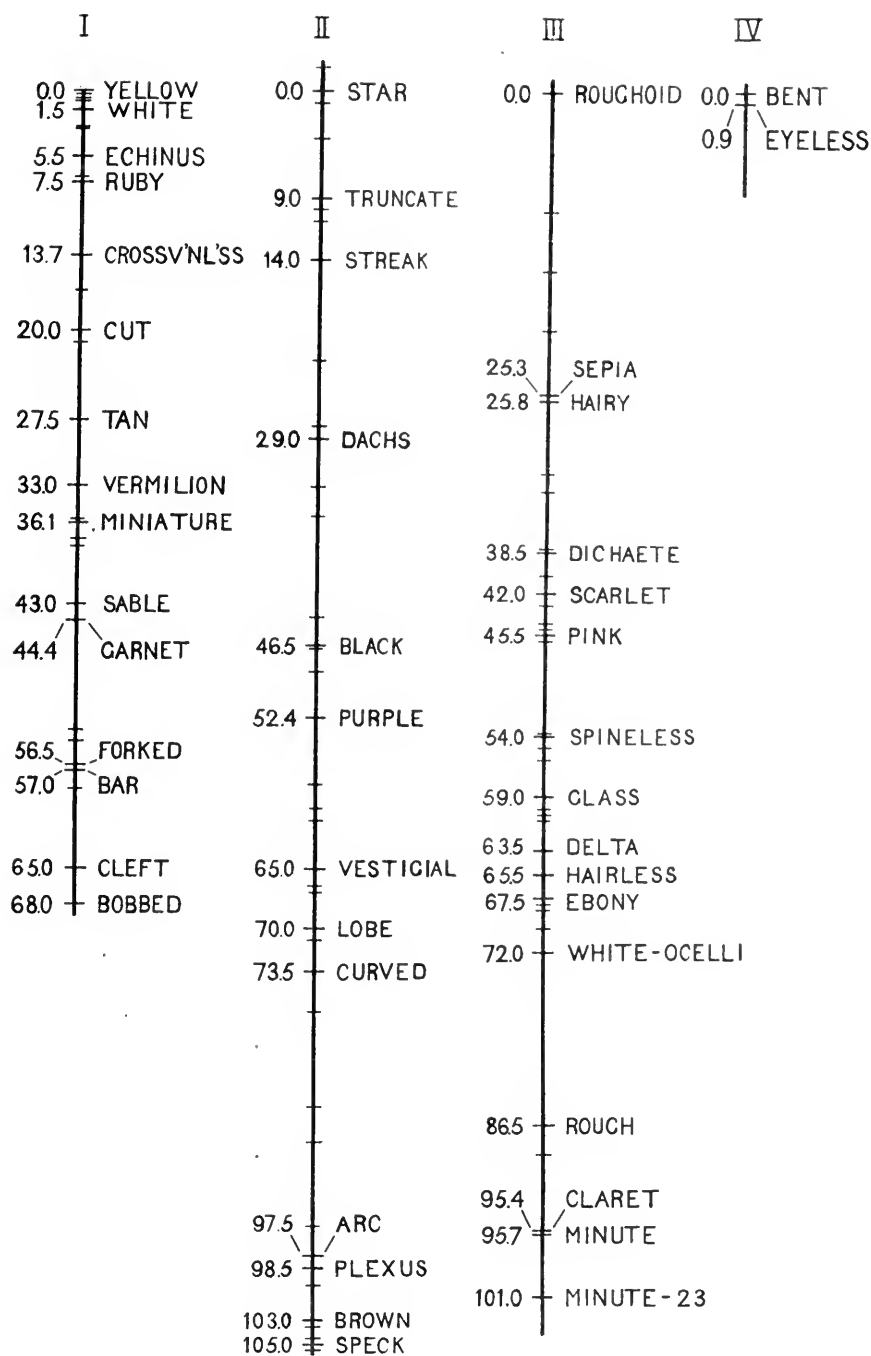


Fig. 2. The four series of linked genes of *Drosophila melanogaster*. The first, I, is the sex-linked series.

tween members of the same pair of linkage groups crossing-over occurs, hence if the chromosomes carry the series of linked genes they must interchange. A very important fact concerning the interchange is shown by genetics. The exchange is precise to a marvellous degree, since it involves an exact interchange of all like loci. Since the genes must be extremely small in order to exist in linear order in the chromosomes, it follows that crossing-over is by no means a casual happening, but the exchange must be carried down somewhere near to the limits of size of the gene or at least to that of the space between the genes, if such spaces are present.

The discovery that certain characters have a somewhat different mode of inheritance from all other characters, and that these characters constitute a linkage group, and that their mode of inheritance is correlated with sex, led naturally to the view that the genes for the sex linked characters are carried by the sex chromosomes (fig. 3). The fact that the male has only one X chromosome (in *Drosophila*) and that he carries only one series of linked factors, while the female, having two X chromosomes, carries two such series of linked factors, furnished strong confirmation of this view. Furthermore, since the male gets his single X chromosome from his mother, he can get sex linked factors only from her (not from his father) if these factors are carried by the X chromosomes. The male does in fact inherit these characters only from his mother. The identification of the sex linked genes with the sex chromosomes has been proven by the exceptional behavior of these chromosomes during the process known as non-disjunction. It is unnecessary here to give the details of this process, they can be found in most modern text-books of genetics. It will suffice for our present purpose to state that it happens, at times, that the Y chromosome (that is normally confined in the male line) gets carried over to an egg, and in consequence an XXY female is produced. She is externally identical with other females, bearing out the evidence that the Y chromosome of *Drosophila* does not carry any of the ordinary genes, but when the eggs of an XXY female pass through the maturation stages, the presence of three chromosomes creates an unusual situation that leads at maturation to irregularities in their distribution. These irregularities are paralleled exactly by what the genetic results show when marked genes are present by which sex-linked characters can be followed. Here, a highly exceptional and rather complicated chromosomal situation gives results that are paralleled by the genetic results. As a matter of fact, the exceptional

genetic behavior was observed first and its study led to the conclusion that three sex chromosomes must be present in the non-disjunctional female. A study of the germ-cells of such females revealed the presence there of the three postulated chromosomes. There are many controls and tests that are possible for this case and these have been made. They leave no doubt as to the genetic result being due to the behavior of the chromosomes.

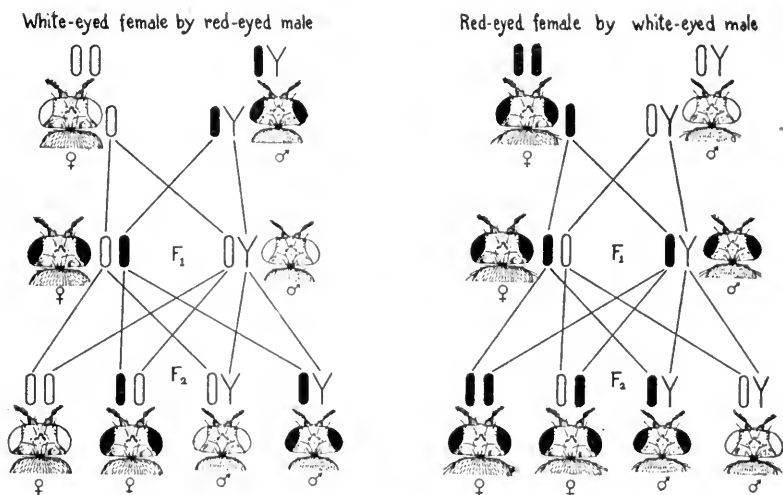


Fig. 3. The inheritance of the sex-linked character, white eyes of *Drosophila melanogaster*. In the part of the diagram to the left a white-eyed female is mated to a red-eyed male. In the part to the right, the reciprocal cross, a red-eyed female is mated to a white-eyed male. In the center, the sex chromosomes are represented. The white (open) chromosome stands for white eyes and the black chromosome for red eyes. The Y stands for the chromosome of that name; it is confined to the male line.

In the process of sex-linked inheritance, referred to above, a mother, if she shows a recessive sex-linked character transmits it to her sons who show it, but if the father carries the sex-linked recessive, neither his daughters nor his sons show it, as is expected from the known behavior of the X chromosomes (see fig. 3). These relations are called criss-cross inheritance. Recently, a case has arisen in which the above relations are exactly reversed—a female with a recessive sex-linked character (yellow) mated to a male with another sex-linked recessive character, produced daughters like herself (instead of sons like herself)

and sons like their father (that carried another recessive). It was apparent from the genetic evidence that the reversal of the ordinary result would be understandable if the two yellow-bearing X chromosomes of such a female were stuck together. If at the same time such a female carried also a Y chromosome then after maturation half of her eggs would retain the double X chromosomes (yellow bearing), and half would retain the Y chromosome (fig. 4). Now if the former kind of eggs should be fertilized by the X-bearing chromosome of the male, they would die, since nearly all three-X individuals perish; but if these XX eggs were fertilized by the Y sperm of the male, a double X female (with Y also) would develop that would have the maternal recessive character (yellow). The other kind of egg, produced by the original XXY mother, contains only the Y chromosome. If this egg is fertilized by a Y-bearing sperm it dies because individuals without an X cannot develop; but if this Y-bearing egg is fertilized by an X-bearing sperm a male (XY) results whose X is derived from his father; and if his father carries a recessive factor he should produce a recessive son like himself. As stated, these genetic results demand a double-X female carrying a Y. Preparations showed positively that two X's (stuck together at one end) and a Y chromosome are present.

In addition to the breeding evidence relating to the X chromosome just given there is also further evidence from the exceptional behavior of the pair of chromosomes of *Drosophila* called chromosome IV. As shown in figure 5a, there is present in the middle of the metaphase chromosome group of the normal fly a small pair of chromosomes that was supposed for a long time to be the bearers of the smallest group of linked genes. There is now both genetic and cytological evidence fulfilling this prediction. A summary of the results will serve to show not only the kind of changes in heredity brought about by the addition or by the loss of a member to this pair of chromosomes but also the general effect of such changes on development.

There are several ways in which the addition of a chromosome may take place—generally it is supposed by primary non-disjunction either in the male or female. The addition of a third chromosome in *Drosophila* (fig. 5b) brings about slight though recognizable departures from the wild type. The eyes of such a triplo-IV fly are smaller, the body color is darker, the wings are narrower. These changes while definite, are so slight that they would ordinarily pass unnoticed. If such a triplo-IV fly is bred to a fly that is normal in type and carries

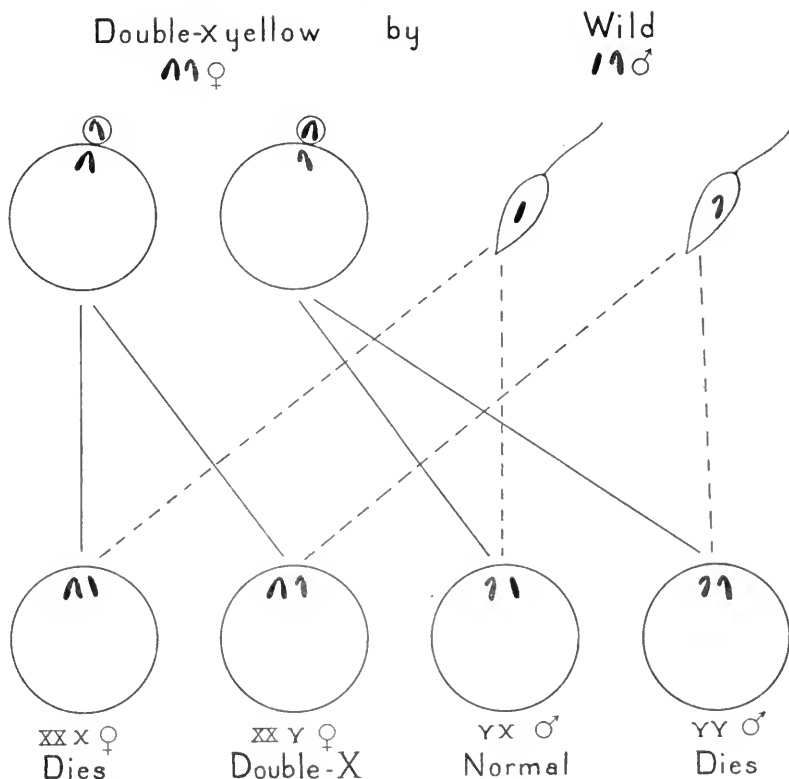


Fig. 4. Diagram to illustrate the two kinds of eggs produced by a double-X, yellow female, and the results of fertilizing them by sperm from a wild type male. The double yellow X-chromosome is represented by the bent loop, and its mate the Y-chromosome by a stippled hook. Four kinds of zygotes are produced (shown in the lower line). Two of these die, namely,  $\text{XXX}$  and  $\text{YY}$ ; two live, namely  $\text{XXY}$  and  $\text{XY}$ . The former is a double yellow female like the parent female, while the other is a male like the father.



Fig. 5. The normal group of chromosomes from the female of *D. melanogaster* is shown in *a*. The group of chromosomes of a haplo-IV female is shown in *b*. The group of chromosomes of a triplo-IV female is shown in *c*.

a recessive factor in one of its IV chromosomes, eyeless for example, the offspring are expected to be of two kinds as shown in the diagram (fig. 6). Half of them are triplo-IV, and half are normal for these chromosomes. Suppose now one of these  $F_1$  triplo-IV flies is back-crossed to an eyeless fly (from stock), the offspring are in the ratio of five wild type to one eyeless instead of equality as in ordinary cases of heredity where a back-cross is made. An analysis of the kinds of germ cells expected after maturation of the eggs or sperm of a triplo-IV

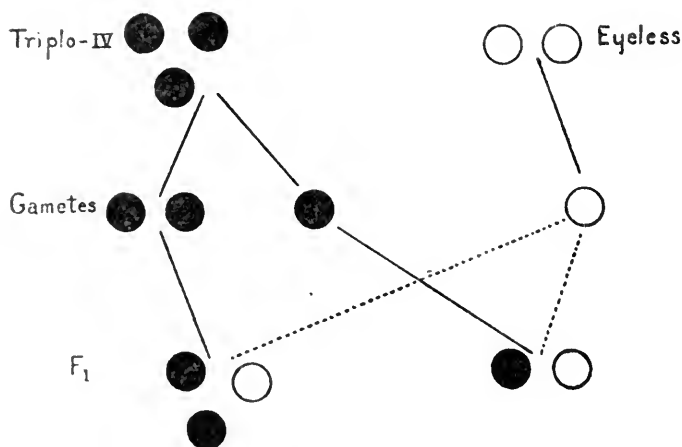


Fig. 6. The result of mating a triplo-IV to an eyeless fly is represented by the chromosomes that are specially involved. The triplo-IV (three black circles), is supposed to carry normal allelomorphs; the other fly, that is eyeless, is supposed to carry the recessive gene for eyeless in the fourth chromosomes (represented here by the open circles). The gametes of these two kinds of flies are represented in the second line, and their recombinations in the third line. Two kinds of individuals result in this first  $F_1$  generation, namely, a wild type that is triplo-IV, and a wild type that is diplo-IV i.e., a normal fly. For a test of the inheritance shown by the former, see figure 7.

individual (fig. 7) explains fully the departure from the Mendelian expectation.

The exceptional genetic behavior shown by the triplo-IV flies based on the theory that they have three IV chromosomes has been confirmed by cytological preparations that demonstrate the presence of three IV chromosomes.

In contrast to the triplo-IV individuals other individuals have been found by Bridges in which there is only one IV chromosome (fig. 5c).

Since only one IV chromosome is present only half of the germ-cells are expected to carry this chromosome, hence if such a fly is bred to a normal fly with two IV chromosomes and homozygous in a recessive IV chromosome factor, eyeless for example, half of the offspring are haplo-IV and these are also eyeless. They come from the egg lacking a IV chromosome, fertilized by a sperm bearing a recessive factor (eyeless) in its IV chromosome. The other half of the offspring are

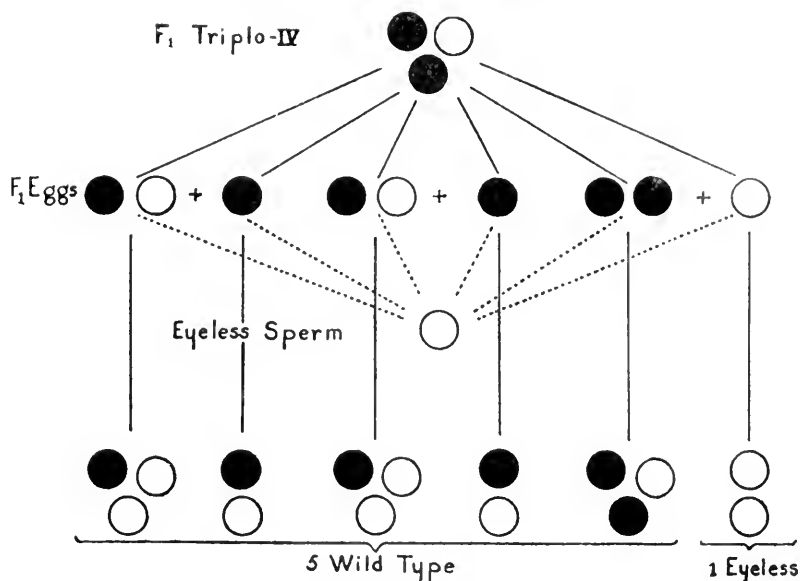


Fig. 7. A back cross of a triplo-IV fly (heterozygous in one chromosome for eyeless; see fig. 6) to an eyeless fly. The three chromosomes of the former are represented at the top, first line; the kind of gametes expected are shown in the second line; the single kind of sperm cell produced by the eyeless fly is represented by the open circle in the middle of the diagram; and in the lower line the six kinds of individuals that result are shown. The expectation is 5 wild type to 1 eyeless.

wild type flies (heterozygous for eyeless). Here then a known recessive Mendelian character appears in half of the first generation offspring. This exceptional result is at once accounted for by the known changes in the number of the chromosomes.

It is probably not a mere coincidence that it is a change in the small IV chromosome that gives these results because its absence is not fatal to the life of the individuals so constituted. No such results

have been obtained for the two large chromosomes, II and III. It would seem, *a priori*, not unlikely that an increase or a decrease in these might so upset the balance, (the effects produced by all the genes) that such an individual would not live. This anticipation has been confirmed by the kinds of offspring produced by still another type of fly in which all of the chromosomes are in threes. These triploids have been found and studied by Bridges both by genetic and by cytological methods. The flies themselves are essentially like the wild type, but a little larger (fig. 8).

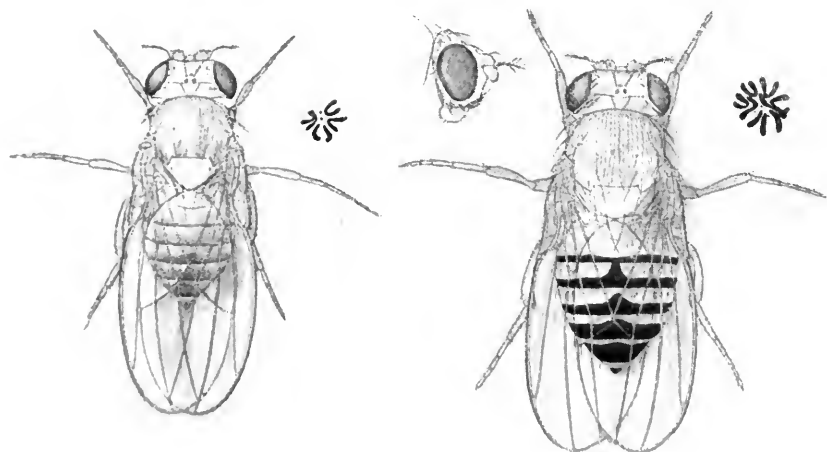


Fig. 8. A normal female (*D. melanogaster*) to the left and a triploid female to the right. The two chromosome groups are shown above and to the right of the respective females.

When the germ-cells of the triploid pass through the reduction period, each set of three behaves in a non-disjunctional way. The result is that there are many combinations of chromosomes possible. Most of these combinations are not viable (after fertilization), and no flies are found in which chromosome II or chromosome III is in triplicate while the rest are in duplicate (normal). It follows that while all the genes may be in twos or threes (or even fours) and the individual containing them may be perfectly viable, an unbalance in one of the sets, if it is large, causes disaster.

Of course it might well be in particular cases that a very small chromosome might contain factors so essential to the life of the individual

that its loss or even its duplication might be fatal. Nevertheless it seems probable, this being conceded, that, in general, when only a few chromosome pairs are present, a change in one set is more likely to work disastrously than a similar change when a large number of chromosomes are present. However, much remains here for the future to decide.

The relations of the large X chromosomes of *Drosophila* (and of many other forms) may seem to stand in contradiction to the statement just made, because one of them is absent from one sex, the male, yet that sex is as viable as the other. We can suppose here that the relations have been gradually brought about by compensating effects elsewhere, i.e., in other chromosomes. In fact this situation is actually utilized, so to speak, to produce two extreme types of individuals within the same species, the male and the female. That the sex chromosome arrangement is intimately interrelated with the effects produced by the other chromosomes is known by the occurrence of intersexes amongst the offspring of the triploid.

*Physiological effects of the genes.* Concerning the ways in which the genes produce their effects genetics has almost nothing to say. There are however certain implications that need to be carefully scrutinized, since it is almost inevitable that speculative views will be advanced concerning the actions of the genes that have little or no foundation in genetics, however probable, or improbable, they may appear from the embryological standpoint.

Mendel used arbitrary symbols (A and a) for the character pairs that he studied. The symbol stood both for the character and for whatever it is in the germ-cells that is there the forerunner of the character. In principle, we use the same scheme today, and since the same symbol stands for both character and gene there is present an opportunity at times for a careless shifting of meaning. In most situations this double meaning of the symbol does no harm, but it has its dangers when, for instance, the variability shown by the character (that may be due to environmental or to modifying genetic factors) is referred to as a property of one gene. Thus, for instance, a rabbit with ears larger than others of its stock might be said to owe its advantage to external factors acting during development or to a different combination of inner factors (genetic) that affect ear length, or to the principal gene affecting ear length being somewhat larger (and therefore more effective) than the average gene. There are well recognized methods by which it can be determined which of these possi-

bilities is responsible for the larger ear of a particular individual. This evidence has shown that results like these are not due to a "larger." gene but either to the environment or to other inner, genetic factors. If the gene were labile, as implied in the last interpretation of variability, the study of heredity would be an extraordinarily complex problem that could be followed only as a mass phenomenon by means of statistical methods. Fortunately there is a large body of information that shows that in heredity we are dealing with individual units of a high order of stability. It is chiefly owing to this that the modern theory of heredity has made rapid progress.

These statements need not be interpreted as dogmatic assertions that the gene is an absolute unchanging unit, that it has no fluctuations, and in this respect comparable to an organic molecule. In the first place, we are not concerned with absolute distinctions. All that we are justified in stating is that by the assumption of stable elements in the germ-material the results we obtain can be explained.

By a stable element we might mean one that maintains a constant size or constitution, or mean only that if it fluctuates quantitatively about a mean, this mean remains the same through countless generations of cell-divisions and generations of individuals. Genetics contributes nothing here, and has in fact no particular interests involved, for either situation would fulfil its requirements.

If our information were more complete, it might be possible from the evidence of crossing-over to discover limiting values for the size of the gene, and find out in this way whether the order of magnitude is such that it comes within the limits of organic molecules. A few tentative estimates of this kind have in fact been made (see Morgan, *The Croonian Lecture*, 1922), but such attempts, at present, are too uncertain to give the kind of information essential to settle the point under discussion.

How the genes produce their effects through the medium of the cytoplasm of the cell, where differentiation becomes realized, we do not know. It has been suggested that the genes are enzymes. Interesting as are such views from a purely speculative point of view, they have at present little to support them. It is, of course, to be hoped that in time the combined attack on this problem of development both by genetics and by experimental embryology and especially by chemistry may lead to the discovery of the physiological action of the genes, but for the present we must confess ignorance.

There is a rather wide-spread interpretation of Mendelian recessive genes as absences. This interpretation began almost immediately with the recognition of Mendel's paper (1900) and has taken the popular fancy. As a pictorial interpretation, or as a kind of symbolism, one need not quarrel with such a view, for it means nothing more than that small *a* means the absence of a character (A). On the other hand if the symbol is taken in a literal sense it may do mischief if in no other way than making an unwarranted interpretation of the internal factorial scheme of Mendelian inheritance. It may not be out of place to give a little space to this question.

It will be advantageous also to consider along with the view that a recessive character means an absence in the germ-material, another view that has grown up beside the other, namely, that a dominant factor is something added to the original complex of factors. The artificiality of these interpretations is obvious. They owe their origin to the common observation that recessives are often due to loss—loss of color, for example—while some dominants appear as additions, enlargements of the original characters. But it is illogical to conclude that there must be corresponding losses or additions of genes in the germ-material, for the results may be due to any kind of change in the germ material that affects the end products of development in such a way that they may be either losses or gains. It must, of course, be granted that a loss of a gene might cause a loss of a character, and that the addition of a gene might cause the increase of a character, yet there is nothing at all in the genetic evidence that supports such an inference and there is something at least that indicates its absurdity. For example, more than 20 dominants are known in *Drosophila*. The genetic evidence indicates that a gene has changed (as seen in its effects) but the number of genes remains the same.

Furthermore, the arbitrary nature of the presence and absence interpretation becomes at once manifest when we recall that the distinction between a dominant and a recessive character is by no means as sharp as implied. On the contrary dominance and recessiveness represent rather the extreme end of a continuous series, rather than alternative conditions. A great many characters are neither dominant nor recessive, but intermediate in the heterozygote. Nevertheless their segregation is as clear as when the character pairs contrasted are strictly dominant or recessive. It would not be a gross exaggeration to say that there is no such thing as complete dominance or recessiveness, but in all heterozygotes the influence of both genes may be pres-

ent, although sometimes difficult to detect, except under exceptional conditions. It follows from this that there are no sufficient grounds for the presence and absence hypothesis as literal interpretation. It is needless to go into further details, but it may be pointed out that there are many cases known in which an addition to a common character—as in some melanic types—is produced by a recessive gene, and where a loss of a character—as in brachydaetyly—is produced by a dominant gene.

When we consider those cases that are known to result from the absence of a group of genes, or by the addition of a group of genes—as in the haplo- or triplo-IV chromosomes of *Drosophila*—there is nothing in the kind of character changes to suggest that one causes losses, the other additions. In fact, the evidence points exactly to the opposite conclusions, for, while the absence of a IV may make the wing longer it also makes it narrower and the presence of an additional IV makes the wing shorter but wider, etc.

*Cytoplasmic inheritance.* There are a few instances known in which self-perpetuating bodies that lie in the cytoplasm produce effects that are inherited. The chlorophyll bodies in plants furnish the best example. Since these, so far as is known, are transmitted only through the cytoplasm of the egg, their inheritance is said to be cytoplasmic. If this form of inheritance were general it would have been frequently met with, but aside from chlorophyll inheritance there are no certain cases of inheritance through the cytoplasm, while practically all the genetic evidence can be interpreted in terms of chromosome inheritance.

The fact that modern genetics has dealt so exclusively with chromosomal inheritance to the exclusion of the cytoplasm has aroused some antagonism from a few embryologists and physiologists, but for somewhat different reasons. The embryologist observes the developmental changes taking place in the cytoplasm in the presence of the entire chromosome complex. He is therefore strongly impressed with the view that the cytoplasm of the cell is the active agent in development. In this he is, of course, entirely right, because his evidence is only concerned with what he can see and not with what takes place behind the scenes. The genetic evidence is the only means we have at present to show that the chromosomes influence what takes place in the cytoplasm.

The physiologist is concerned with processes going on in the cell, but has no means of finding out at present how far the chromosomes may or may not have affected the process. He is, however, as a rule

sufficiently broad-minded to concede at least that any change in the complex of factors present in a cell may affect the end result. Genetics supplies this evidence; it is not concerned with the processes that take place either before or after the postulated change—at least only secondarily interested. Whether or not genetics may in time develop new methods that make possible an approach to some of the problems that at present belong to the field of experimental embryology and physiology remains to be seen, but there is no conflict at present between these studies and no grounds for prejudice. The small amount of prejudice that exists is due to a misconception of the limitations that the geneticist places on his own work.

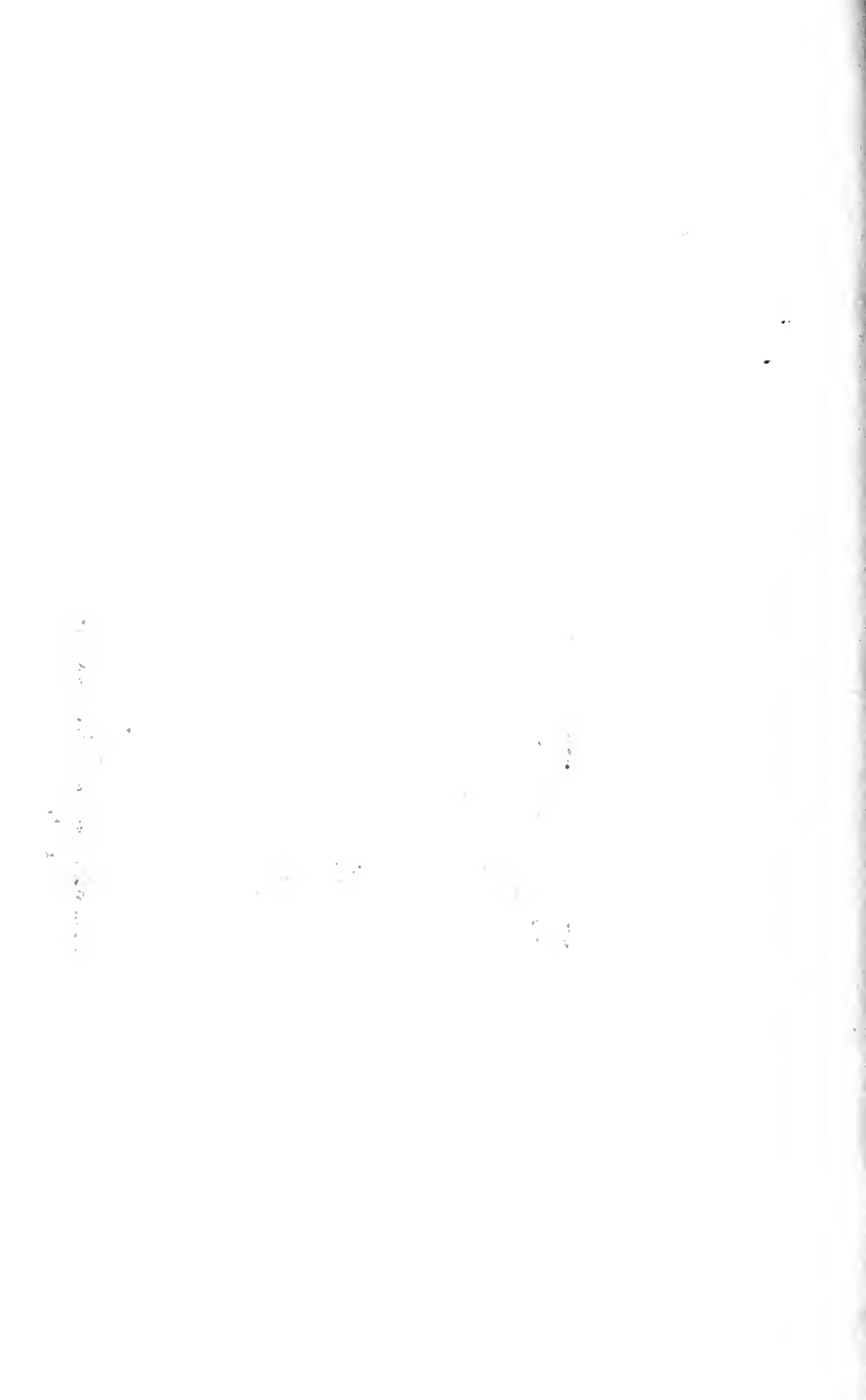
The question remains nevertheless as to how discrete particles in the chromosomes can produce effects on the cytoplasm of the cell of such a sort as to determine the developmental process and through it the later physiology of the cell. Whether the genes are active all the time or only under specific conditions we do not know, but at least it may be granted that there is no evidence to indicate that all of them are not always at work. How they work we do not know. Several suggestions have been made that are purely speculative or fictitious. The only important contribution that genetics has made in this field may be illustrated by an example. If a red-eyed female *Drosophila* that has a red-eye-producing gene and a white-eye-producing gene (i.e., if she is heterozygous for white) is mated to a pure white male, half the daughters are red-eyed, half white-eyed. The latter are indistinguishable from white-eyed females, all of whose parents for generations have been white-eyed. This means that the presence of a red-producing gene in the egg of the mother (and in the generations of oöcytes that by division have produced the egg) has had not the slightest effect on the character of the white eye of those of her daughters that have two white genes. This evidence is explicit in showing that even if the presence of the red-producing gene in the egg has affected the cytoplasm of the egg up to the time of maturation, that effect is done away with by the introduction of a white-producing gene at the beginning of embryonic development. It is this kind of evidence from genetics that shows the minor rôle in inheritance played by the cytoplasm. It is needless to point out, perhaps, that the evidence in no sense indicates that the cytoplasm may not be as important for the development as are the chromosomes—only it is under their influence.











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